

THE EFFECTS OF CERTAIN DRUGS
ON TISSUE RESPIRATION OF THE LIVER

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ABSTRACT

The etiology of cirrhosis is little understood due to a paucity of information concerning the biochemical phenomena involved. It was thought that tissue respiration studies might provide some pertinent information. An investigation was therefore undertaken to determine the effects of corticotropin, cortisone, alcohol and methionine upon the liver respiration of normal and carbon tetrachloride-treated rats. Carbon tetrachloride was administered alone and concurrently with each of the drugs, and each drug was administered after a series of carbon tetrachloride injections had been discontinued. In addition, each drug was administered to normal animals. Administration of the drugs and carbon tetrachloride extended over a period of 15 weeks. QO_2 values were determined using liver slices in Krebs' Medium III (KMIII) and in oxygen (HM flasks). Sections were taken from representative animals in each group for histological examination to correlate with the results obtained by tissue respiration.

Liver respiration of normal animals increased significantly with the age of the animal. Corticotropin, alcohol, methionine and carbon tetrachloride had no significant effect upon liver respiration. In contrast, cortisone significantly depressed liver respiration. Histological examination

indicated that carbon tetrachloride produced an alteration in the histological pattern which advanced in the direction of a cirrhotic lesion. The pronounced regenerative activity which enabled the liver to function normally was reflected by the QO_2 values.

Corticotropin, cortisone, alcohol and methionine, each administered concurrently with carbon tetrachloride, caused a depression in liver respiration. In addition, the histological distortion was more severe than when carbon tetrachloride was administered alone. The levels of depression produced by alcohol and methionine were much lower than those produced by cortisone and corticotropin.

When carbon tetrachloride was administered for 4 weeks and then discontinued, the liver respiration appeared normal in HM flasks, but was significantly below normal in KMIII. Administration of the drugs following cessation of carbon tetrachloride injections produced similarly equivocal results.

It is suggested that the Huston-Martin technique has several advantages over the conventional Warburg technique in pharmacologic investigations at the cellular level.

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THE UNIVERSITY OF ALBERTA

THE EFFECTS OF CERTAIN DRUGS ON
TISSUE RESPIRATION OF THE LIVER

A DISSERTATION
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
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DEGREE OF MASTER OF SCIENCE

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by

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1. INTRODUCTION

The liver, which is the largest gland in the body, plays an important role in the maintenance of metabolic equilibrium. The metabolic abnormalities occurring in liver failure touch on every aspect of human physiology. The term "cirrhosis" was first coined by Laennec in 1819. By the turn of the 20th century, many of the manifestations of cirrhosis and allied diseases of the liver were recognized, and during the past 2 decades, the production of liver lesions in experimental animals by dietary deficiencies, hepatotoxic agents and other procedures, has brought a new understanding of the pathological and physiological changes which underlie the pattern of signs and symptoms.

A wide variety of unknown etiological factors contribute to the development of liver disease. By relatively simple techniques it is possible to produce a cirrhotic lesion in experimental animals and to study the effects of various drugs on this lesion. Heretofore the investigations undertaken have been carried out by means of biochemical and

histological methods. There appears to be a paucity of research in the field of tissue respiration, and the cirrhotic lesion remains an enigma. By investigating at the cellular level, it may be possible to obtain a better understanding of the complex mechanisms underlying liver disease.

II. CHEMISTRY AND GENERAL PHARMACOLOGY OF DRUGS USED IN THE INVESTIGATION

A knowledge of the chemistry and the general pharmacological activities of the drugs used is necessary for an understanding of the mechanism of action of the drugs on the biochemical phenomena being investigated and the interpretation of the observed effects.

1. Ethyl Alcohol

Ethyl Alcohol, $\text{CH}_3\text{-CH}_2\text{-OH}$, otherwise known as ethanol, Spiritus Vini Rectificatus, and methylcarbinol, is a transparent, colorless, mobile, volatile liquid with a slight characteristic odor and burning taste (1). It is miscible with water, ether and chloroform, boils at 78.4°C . and has a specific gravity of 0.79 at room temperature. Both chemically and physiologically, ethyl alcohol belongs to the group of aliphatic narcotics.

Alcohol first depresses the higher inhibitory centers in the cerebral cortex. Descending depression causes diplopia, ataxia and flaccid reflexes.

Alcohol does not affect respiration markedly except in doses sufficiently large to cause severe intoxication, when respiration may be seriously depressed. The cardiovascular system also shows only minor effects from alcohol.

Moderate doses cause vasodilatation, especially of cutaneous vessels, but the direct action of alcohol on blood vessels is insignificant.

Although in low concentrations, alcohol has been used as a stimulant or appetizer, higher concentrations produce a definite inflammatory response in the gastrointestinal tract with interference in the function of the gastric mucosa. Alcohol exerts a diuretic effect on the kidney by causing a decrease in renal tubular reabsorption of water. Alcohol has no effect on skeletal muscle activity and produces some relaxation of tone of smooth muscle(2,3).

Alcohol is rapidly absorbed from the stomach, small intestine and colon, as well as through the lungs. After absorption, it is distributed throughout all the tissues as well as the cellular and extracellular body fluids. Oxidation of alcohol begins immediately upon absorption and 90 - 98 per cent of alcohol entering the body is completely oxidized. The

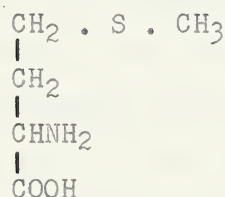
liver is the primary site for the initial oxidation of alcohol. It contains the enzymes ethanol and aldehyde dehydrogenases, xanthine oxidase and catalase. Ethanol dehydrogenase catalyzes the conversion to acetaldehyde; flavoprotein and aldehyde dehydrogenases then catalyze the oxidation of acetaldehyde to acetic acid or active acetyl, which are converted to carbon dioxide eventually via the tricarboxylic acid cycle.

Alcohol is excreted mainly through the kidney and lungs, but can be detected in other secretions.

The pathological effects attributed to chronic ingestion of alcohol are often, in reality, the result of vitamin deficiencies incident to poor food intake or faulty gastrointestinal function of the alcoholic (4). Cirrhosis of the liver occurs in 8 per cent of chronic alcoholics in contrast to 1 per cent of abstainers and temperate drinkers, although experimental results indicate that cirrhosis is not due to a direct toxic action of alcohol, but is due to contributory unknown etiological factors.

2. Methionine

Methionine, a sulfur-containing essential amino acid, occurs as white crystalline platelets or powder. Chemically, DL-Methionine is α -Amino- γ -methylmercaptobutyric Acid.



The L form is the naturally occurring isomer. One gram of methionine dissolves in 30 ml of water. Methionine is soluble in dilute acids and in solutions of alkali hydroxides; very slightly soluble in alcohol; and almost insoluble in ether(1,6).

Methionine plays an important role in animal nutrition as a source of both labile methyl groups and sulfur necessary for normal metabolism(2). The value of methionine as a lipotropic agent in the protection of animals against liver injury by halogenated hydrocarbons will be discussed later.

3. Corticotropin (ACTH, Adrenocorticotropin)

Corticotropin is a preparation of the principle or principles derived from the anterior lobe of the pituitary gland of slaughterhouse animals which exert a tropic influence on the adrenal cortex. It stimulates the adrenal cortex to secrete its entire spectrum of hormones. Experimental evidence suggests that hydrocortisone is the chief component in the adrenocortical secretion, although important quantities of aldosterone, cortisone and corticosterone are elaborated. Hormonal effect can be exerted only if a functioning adrenal cortex is present and the physiologic and metabolic effects are due to the adrenal corticosteroids elaborated. Corticotropin is utilized rapidly in the body; its effect rarely exceeds 6 hours. The activity of corticotropin is destroyed by proteolytic enzymes of the gastrointestinal tract, and thus the preparation is ineffective when given orally.

It is now well established that in response to stress the secretory activity of the adrenal cortex is greatly increased, and that this important response is mediated by corticotropin. There are several intriguing theories regarding

the nature of the regulatory mechanism which controls the rate of discharge of corticotropin. A number of discussions on this subject are available (2,3).

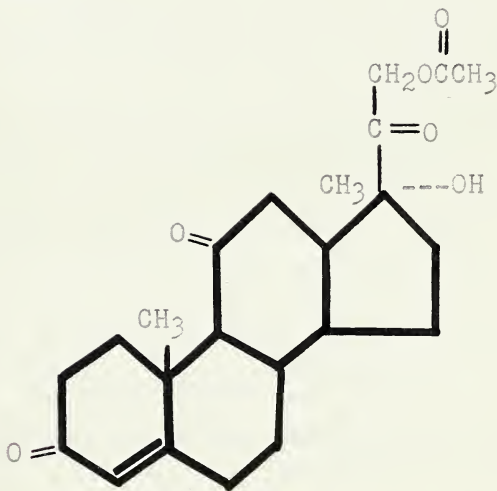
The injection of corticotropin or its increased secretion in response to stress results in an increase in weight of the adrenal cortex. In addition chemical changes occur in the adrenal cortex which are believed to result from the increased rate of synthesis and release of adrenocortical steroids. The content of ascorbic acid and cholesterol are both depleted following a single injection of corticotropin. Similar changes occur in the adrenal glands of intact animals subjected to stress, but not in the glands of hypophysectomized animals. It is believed that cholesterol disappears because it is a precursor of adrenocortical steroids, but the explanation for ascorbic acid depletion is unknown (2).

The administration of corticotropin is accompanied by many of the same undesirable hormonal effects as follow the prolonged use of cortisone. It does not cause involution or atrophy of the adrenal cortex, as does cortisone,

but the sudden cessation of corticotropin administration may be followed by a period of depressed adrenocortical function. Prolonged use of the drug induces the clinical picture observed in Cushing's syndrome.

4. Cortisone

The cortisone used in this investigation was in the form of the acetate and has the following formula:



11 - Dehydro - 17 - hydroxycorticosterone -
21 - acetate.

Cortisone acetate is a white, or practically white, odorless, crystalline powder. It is slightly soluble in alcohol and practically insoluble in water.

Cortisone is one of the carbohydrate-regulating hormones of the adrenal cortex concerned in glucogenesis which are termed glucocorticoids.

When injected into adrenalectomized animals the glucocorticoids maintain life and resistance to various forms of stress ordinarily lethal to the unprotected adrenalectomized animal. The glucocorticoids affect fat, protein and carbohydrate metabolism promoting glucogenesis, hyperglycemia, glycosuria, and negative nitrogen balance unless adequate protein is supplied. They inhibit the activity of the lymphatic system. They induce mild sodium retention and potassium excretion, but large doses given over a period of several days may alter the electrolyte balance profoundly.

Glucocorticoids inhibit the production of corticotropin by the pituitary and depress the function of the adrenal cortex. Continued use causes atrophy of the thymus and varying degrees

of atrophy of the adrenal cortex. When glucocorticoids are administered to patients over extended periods they may cause widespread physiologic and metabolic effects resembling those encountered in Cushing's syndrome.

It is now well established that under conditions of stress the secretory activity of the adrenal cortex is greatly augmented. The response of target cells and organs to the secretion of the adrenal gland and to the injection of adrenocortical steroids may vary both qualitatively and quantitatively, depending upon the activity and the environment of the organism. Although the fundamental cellular mechanism of action of the adrenocortical secretion is not known, a number of basic cellular reactions are known to be modified. For example, wound healing and the normal inflammatory reaction to infection are retarded; alterations occur in the collagen tissues of the body; and the normal reactions of the organism to certain stresses is suppressed. The reactivity of mesenchymal tissue and the permeability of cellular membranes is altered, possibly by an inhibiting effect on the hyaluronidase system.

5. Carbon Tetrachloride

Carbon tetrachloride, CCl_4 , is a colorless, mobile, non-inflammable liquid. It is very slightly soluble in water and miscible with alcohol.

Carbon tetrachloride has narcotic and anesthetic properties resembling those of chloroform. The drug is slowly absorbed from the alimentary tract and is somewhat cathartic. Both inhalation of the vapors and oral ingestion may result in acute and chronic poisoning (2). The early symptoms of acute poisoning result from central nervous system effects and consist of headache, dizziness, stupor and unconsciousness. Further exposure is followed by progressive depression and medullary paralysis. Depression of the myocardium and vasomotor center may result in cardiovascular collapse. Excessive oral ingestion results in abdominal pain, accompanied by nausea, vomiting and diarrhoea. Following recovery from the immediate effects of carbon tetrachloride, a patient may eventually exhibit the late toxic effects of acute poisoning which are evidenced by the hepatotoxic and nephrotoxic actions

of the drug. Repeated exposures to carbon tetrachloride result in subacute or chronic poisoning. The liver is the organ chiefly affected, although renal damage, hypertension, severe dermatitis, conjunctivitis and bronchitis have been reported. The factors which influence the hepatotoxic action of the drug will be discussed later.

III. LITERATURE SURVEY

There is an extensive literature on the clinical aspects of cirrhosis. This will not be reviewed here. The discussion will be confined to experimental cirrhosis except where clinical reports are particularly pertinent to the experimental findings of this work.

1. Carbon Tetrachloride in Experimental Cirrhosis

The hepatotoxic action of carbon tetrachloride has been recognized for some time, with the result that the drug has been used extensively in the investigation of experimental cirrhosis.

The action of carbon tetrachloride on the liver involves three progressive stages (7). At the first stage, carbon tetrachloride enters the centrolobular cells (8). The fact that only the centrolobular cells are involved suggests that only these cells are functionally capable of admitting the injurious agent. The second step is manifested by the rapid swelling of the cells and dispersal of their basophilic bodies, loss of glycogen and accumulation of sudophilic lipids. Carbon tetrachloride is a lipid solvent and may

attack the lipid component of the intracellular membranes, resulting in changes in the permeability of cell membranes with imbibition of fluid.

Finally in the third stage, the mitochondrial membranes are attacked, possibly in the same manner.

The details of the sequence of events that takes place after carbon tetrachloride administration have been described by a number of workers (7,9,10, 11, 12, 13, and 14). The degree of damage to the liver depends upon the size of the dose, the frequency of administration, and the number of doses (14 - 20). Rats receiving repeated doses of carbon tetrachloride differ in the recuperative power of their livers. Cameron and Karunaratne (16) report that rats given 6 to 10 doses of carbon tetrachloride at short intervals show appearance of early monolobular cirrhosis which disappears rapidly following cessation of injections, with the liver returning to normal within 10 days. With longer periods of exposure the liver shows less and less tendency to assume a normal appearance when the poison is discontinued. When there is time for recovery after each dose, carbon

tetrachloride can be administered almost indefinitely without producing any permanent alteration.

There are two stages in the development of carbon tetrachloride cirrhosis, (a) a pre-cirrhotic reversible stage with histological features indistinguishable from actual cirrhosis, and (b) an irreversible cirrhotic stage with a finely or coarsely granular liver. Restoration is due mainly to liver cell proliferation.

Post et al. (17) report that successive injuries to the liver, separated by periods of healing, are associated with an increasing deficit in its restorative capacity. A single injury produces profound biological changes in the organ long after histological restoration is achieved, and this altered biological state is manifested in the delayed healing response to successive injuries (20).

The liver shows amazing regenerative capacity. Islami et al. (18) found that growing, male, albino rats with advanced stages of cirrhosis could completely recover from the cirrhosis using the residual cirrhotic liver left after 70 per cent hepatectomy. In contrast, however, attempts by

Mann et al (19) and Cameron and Karunaratne (16) to obtain regeneration of normal liver by partial hepatectomy in cirrhotic animals were unsuccessful.

Investigators agree that an early effect of carbon tetrachloride is swelling of the centrolobular hepatic cells, apparently by imbibition of fluid (9, 12, 21, and 22). The mechanism by means of which this effect is produced and its role in subsequent changes are not clear and have been variously interpreted. The puzzling fact that this swelling plus subsequent necrosis occurs in the central zone of the lobule, instead of the periportal zone where the cells are first exposed to the circulating poison, has been interpreted by Glynn and Himsworth (23) as a secondary and indirect action of carbon tetrachloride. They suggest that a swelling of the hepatic cells produces an ischemia of the organ which in turn brings about a deficiency in the oxygen supply of the centrolobular cells. Necrosis is the result of this anoxia. In contrast, Myren (12) proposes that hepatic cells, especially in the midzone, are directly attacked by carbon tetrachloride. Then the swelling of these cells impedes circulation, and the injury to the centrolobular cells follows. Daniel, Prichard and Reynell (24) have questioned the involvement of anoxia in carbon tetrachloride-

induced necrosis on the basis that there is no evidence of impairment of circulation of blood in the liver after carbon tetrachloride administration. Leduc and Wilson (7) have shown that carbon tetrachloride affects cell membranes and the membranes of the endoplasmic reticulum with subsequent dispersal of cytoplasmic basophilic bodies, diminution of glycogen, and accumulation of neutral fat. They assume that these three phenomena are reversible and do not lead to death of the cell. The initial loss of the aggregates or clumps of basophilic material from the centrolobular cells was first interpreted as a diminution in the amount of ribonucleoprotein (9,10, and 25). However, it has been suggested that there may be simply a dilution effect brought about by the entrance of water into the cells with a resulting dispersal of the aggregates (20,21, and 22), and this theory has been confirmed with electron microscopy.

The loss of glycogen and accumulation of fat in the liver can be interpreted as expressions of disturbed metabolic activity. Although the significance of these changes is not known, the loss of glycogen from the preneurotic cells might result from anaerobic glycolysis, and the

subsequent loss from the surviving cells as a result of a drain on liver reserves for blood sugar maintenance and for energy requirements. The occurrence of glycogen depletion and excess fat accumulation in the centrolobular zone indicates that there is an injury to these cells which is sufficient to bring about a temporary metabolic disturbance, but not great enough to cause death of the cell (7).

Enspherulation and swelling of the mitochondria occur in response to a great variety of types of cell injury, and are probably due to the imbibition of fluid resulting either from an increase in the permeability of the mitochondrial membranes or from a change which makes the matrix within the mitochondria more hydrophilic. (22). Christie and Judah (22) indicate that carbon tetrachloride damage does not produce an inhibition of specific enzymes but a disorganization of the topographical relationships of the enzymes, which results in a loss of their integrated activity. They propose that carbon tetrachloride acts directly on the cell by increasing the permeability of the mitochondria which in turn brings about a loss of enzymic co-factors and a consequent disruption of organized enzymatic control of the metabolic activities of the cell. These changes bring about

the death of the cell. The mitochondria and their constituent enzymes, therefore, seem to be important targets for the injurious effect of carbon tetrachloride. Dianzani (15) reports that fatty infiltration of the liver obtained by treatment with carbon tetrachloride results in a decrease in the concentration of adenosine triphosphate (ATP) in the mitochondria as a consequence of increased adenosine triphosphatase (ATPase) activity. He assumes that depletion of ATP occurs before fatty infiltration. Calvert and Brody (26) found that stimulation of ATPase is apparent only at high concentrations of carbon tetrachloride and may not be a factor in-vivo.

2. The Effect of Drugs on Experimental Cirrhosis.

(a) Miscellaneous

A variety of compounds have been shown to exert at least a partial protective action against carbon tetrachloride injury. Drugs which have been reported to have such an action include xanthine (27),

sodium thioglycollate, glutathione, sodium thiomalate and cysteine (28); aureomycin, cortisone and folic acid (29); vitamin B₁₂ (30, 31, and 32); sulfaguanidine (7); and ethylene-diamine-tetra-acetic acid (EDTA) (26).

(b) Dietary Factors

Another factor which influences the hepatotoxic action of carbon tetrachloride is protein. Dietary deficiencies of choline, essential amino acids, essential fatty acids and inositol can under certain conditions give rise to an accumulation of liver fat. Choline has received considerable attention from a number of workers, but its role in normal or pathological liver is not clear (5, 33, 34 - 41). Methionine also has been extensively investigated (5, 42 - 55). Tucker and Eckstein (56) found that methionine was effective in preventing fatty livers in rats consuming choline deficient diets. The lipotropic effect of methionine was confirmed in several laboratories (57, 58, and 59), and is attributed to its ability to contribute methyl groups for choline synthesis.

The value of lipotropic agents, more specifically methionine, in the treatment of carbon

tetrachloride poisoning is not established. Goodman and Gilman (2) postulate that chlorinated hydrocarbons inactivate hepatic sulphydryl enzyme systems and that the liver is more susceptible when the diet lacks available sulfur. They feel that although sulfur-containing amino acids can protect against the enhanced susceptibility to liver damage that occurs in protein-deficient states, there is little evidence that the compounds exert a beneficial action after the organ has been injured. There are several clinical reports which claim that the administration of methionine exerts a favorable effect in toxic hepatitis induced by carbon tetrachloride, and in contrast, there are reports that while methionine and choline are highly effective in the prevention of experimental injury in animals, they are ineffective in the therapy of acute liver disease in humans(40, 50). Cohen et al (53) found that once cirrhosis is fully established, addition of lipotropic factors such as choline or methionine to low casein diets did not bring about adequate repair or regeneration of the liver. Rees and Kline (38) studied the metabolism of liver slices from rats on a choline deficient diet, and found that the addition of choline increased the QO_2 of slices of fatty liver more than it did the QO_2

of control slices. Munro and Mukerji (55) found that the addition of excessive amounts of individual amino acids to the diets of experimental animals gave rise to amino acid imbalances and toxicities.

(c) Alcohol

It is well known that the ingestion of alcohol at or near the time of exposure to carbon tetrachloride greatly predisposed the patient to the serious effects of the chemical. Carbon tetrachloride is soluble in alcohol and the ingestion of alcohol and carbon tetrachloride simultaneously may tend to increase the absorption of the carbon tetrachloride. If exposure was by inhalation, and if alcohol was also being excreted at the same time via the lungs, or was present in the blood in significant concentration, it seems possible that the absorption of carbon tetrachloride might also be enhanced (60). However, because the ingestion of alcohol after exposure to carbon tetrachloride also appears to predispose to serious injury, it may be that it is not an enhancement of absorption of carbon

tetrachloride by alcohol which is responsible. It is known that carbon tetrachloride can be oxidized to phosgene, and that phosgene in-vitro can condense with ethanol to form ethylchloroformate. Guild et al (60) speculate that possibly this sequence of events can occur in-vivo. In the absence of ethanol, phosgene can condense in-vitro with ammonia to form urea, a harmless substance. Zieve (61) has noted a rise in serum lipids, including cholesterol, phospholipids and neutral fat in alcoholic fatty liver. Griffith (62) postulates that hepatic disease results from the combination of excessive alcohol intake and dietary deficiency. The high caloric content of alcohol may increase the demand for lipotropic substances and thereby lead to pathological changes in the liver despite an otherwise adequate diet.

(d) Cortisone and Corticotropin

Studies on the effect of cortisone and corticotropin on the liver have in recent years attracted considerable attention, both of clinicians and of experimental workers observing the progress of carbon tetrachloride-induced cirrhosis. The results of these studies are,

however, conflicting. Aterman (63,64) and Cavellero et al (65) noted a reduction in the fibrous content of the livers of rats if cortisone was given simultaneously with carbon tetrachloride, although fatty degeneration was not affected. Diengott and Ungar (66), on the other hand, observed that cortisone therapy aggravated the cirrhosis thus induced. The hepatic lesions were uniformly more severe in all animals treated with cortisone than in the control rats. Aterman and Ahmad (67) reported considerable deterioration of liver function in such rats. Vorhaus and Vorhaus (29) found that rats pretreated with cortisone showed obvious protection against the intoxicating effects of carbon tetrachloride, although no apparent effect was observed with regard to regeneration or fatty infiltration. They assume that cortisone served to protect the hepatic cells at the cellular level, decreasing inflammation and inhibiting fibroblastic activity.

Brown and associates (68), in their clinical studies, noted reduction in fibrosis and fatty infiltration after corticotropin. Zoeckler (69) observed that cortisone resulted in a beneficial effect upon the plasma protein concentration in cirrhotic patients, but there was no effect on the other liver functions or on the hepatic

architecture. Fatty metamorphosis did not occur as a result of cortisone therapy. In contrast, Williams and Flink (70) tried corticotropin on 10 patients with chronic liver disease and were doubtful whether any patient benefited significantly. Wahi et al (71, 72), interpreting ascorbic acid levels as an indication of relative adrenal activity, noted that the adrenals become involved in the chain of events produced by carbon tetrachloride. The reversible stage of cirrhosis is associated with a normal functioning adrenal, or at least one that can be induced to further activity when subjected to stress. With the onset of irreversibility, the adrenal cortex is noted to be functionally damaged. Possibly the altered capacity of the liver cells to deal with the steroid hormones results in a disproportionate collection of these substances within the body. Such an accumulation would act on the anterior pituitary and hamper its capacity to elaborate corticotropin. In addition, carbon tetrachloride may be toxic to the pituitary itself. These same authors found that cortisone helps in the regression of cirrhosis during the phase when the process is naturally reversible. At a later stage no appreciable regression is caused.

They felt that cortisone helps to undo only certain earlier changes in the maturation of collagen. Once mature fibrous tissue is laid down, cortisone is ineffective.

Bach et al. (73) reported changes in the activity of extracted enzyme systems after treatment with adrenal corticoids. Dunn et al. (74) found that administration of cortisone to mice produced only temporary modifications of the cellular components of the liver which indicate rapid adaptation of this tissue to cortisone. Ashmore et al. (75) report that the adrenal cortical steroids do not affect the rate of glucose phosphorylation and the metabolism of glucose via the phosphogluconate oxidation pathway. The only effects observed were those of increased gluconeogenesis and decreased lipogenesis. Pessar and Hessing (76) applied large dosages of cortisone in six cases of hepatic coma, and although all the patients eventually died of various complications of cirrhosis, they felt that a continued trial of cortisone in hepatic coma is warranted.

Wool and Goldstein (77) felt that 3 factors are implicated in the process leading to the production of a fatty liver, the presence of

cortisone, epinephrine, and a secretion of the anterior pituitary. They found that fat mobilization and consequent fatty liver can occur in the absence of the hypophyseal mobilization factor provided the animal is maintained on cortisone and epinephrine. In the adrenalectomized animal, the ability to develop fatty liver is restored to normal levels with cortisone and epinephrine. But in the hypophysectomized animal, restoration is not complete. Therefore it would appear that the anterior pituitary plays a role in fat mobilization.

There exists a rather extensive literature on the influence in-vitro of the steroids on tissue metabolism. According to these studies (78,79), most of the steroids, even the biologically inactive ones, depress the metabolism and the enzymatic activity of the tissues to which they are added. These results are not in agreement with the observations in-vivo, and many authors consider these in-vitro activities with their uniformity of depression and absence of specificity not as a physiological but rather as a toxic side effect. According to Umbreit (80), Gold and Sturgis (81) and Schwartz et al (82), factors

such as cellular integrity and a latency period, which are only present in in-vivo experiments, are necessary to establish the true effect of the steroids. Lacroix and Leusen (83) presented data which support the view of a specific action of cortisone on tissue metabolism in-vivo which includes, among other characteristics, a sex and tissue-linked specificity. Cortisone produced a stimulation of the diaphragmatic and a depression of the myocardial respiration. Cortisone seems to antagonize thyroxine activity at some tissue levels (heart) while at others (diaphragm) it displays a cumulative or additive activity.

By which mechanisms and at which level of the cellular metabolism the adrenal steroids and the adrenocorticotrophic hormone exert their regulatory influences on metabolism remains unanswered. They may act by modifying the activity of specific enzyme systems or by altering the permeability of the target cells. Each mechanism, or a combination of the two, may be the basis for hormone action in different instances. Further investigations on steroid-hormone-enzyme relationship and on cellular permeability are needed to enlarge our insight into the problem.

IV. STATEMENT OF THE PROBLEM

Although there has been extensive histological examination of the hepatotoxic action of carbon tetrachloride and some biochemical investigation, there has been little attention given to oxygen utilization by the liver. Since tissue respiration studies might be expected to yield basic information helpful to an understanding of the toxic livers, the oxygen utilization of normal tissue was compared to that of poisoned tissue. The effect of alcohol, methionine, cortisone and corticotropin on the respiration of liver from normal and carbon tetrachloride treated animals was also investigated.

Standard methods of Warburg examination involve suspension of the tissue in liquid medium. Huston and Martin (84) have proposed a technique whereby the tissues to be studied are excised, spread on fibre glass mats and placed in an atmosphere of oxygen in a special type of Warburg flask. It is felt that this technique would more closely approach the in-vivo situation. This procedure has been used successfully in

pharmacologic studies (85,86,87). It was the further purpose of this investigation to compare the results obtained with the tissue in liquid medium and suspended in oxygen.

V. EXPERIMENTAL

1. Method of Handling Animals and Drugs

The drugs were administered as follows:-

- (a) Carbon tetrachloride: 0.1 cc in 0.1 cc olive oil by subcutaneous injection on alternate days.
- (b) Corticotropin (Duracton, Nordic Biochemicals): 0.4 international units by subcutaneous injection, daily.
- (c) Cortisone (Cortone Acetate, Merck & Co. Limited): 1 mg by subcutaneous injection, daily.
- (d) Alcohol:- 10 per cent solution in drinking water.
- (e) Methionine:- approximately 200 mg per rat daily by dissolving in the drinking water.

Male albino rats of the Wistar strain weighing 125 - 150 grams were separated into 15 groups containing 15 animals each.

The several groups were treated with the above drugs as follows:-

Group 1. Normal rats which served as controls.

Group 2. Carbon tetrachloride.

Group 3. Carbon tetrachloride for one month and

thereafter discontinued.

- Group 4. Corticotropin.
- Group 5. Corticotropin and carbon tetrachloride.
- Group 6. Carbon tetrachloride for one month,
then discontinued and corticotropin
given.
- Group 7. Cortisone.
- Group 8. Cortisone and carbon tetrachloride.
- Group 9. Carbon tetrachloride for one month,
then discontinued and cortisone given.
- Group 10. Alcohol.
- Group 11. Alcohol and carbon tetrachloride.
- Group 12. Carbon tetrachloride for one month,
then discontinued and alcohol given.
- Group 13. Methionine.
- Group 14. Carbon tetrachloride and methionine.
- Group 15. Carbon tetrachloride for one month,
then discontinued and methionine given.

2. Determination of Tissue Respiration

(a) Apparatus and Solutions Used

In order to measure the respiration of tissues in contact with oxygen, it is necessary to have the samples suspended in such a manner

as to ensure maximal contact of the tissue with the gas. The slices of tissue were spread out evenly on fibre-glass mats and placed in wide mouthed flasks designed by Huston and Martin (84).

Since it was desirable to compare the respiratory rates of tissues suspended in oxygen with those found by more standard procedures, samples of each liver were also investigated in liquid medium. Regular Warburg flasks were used for this purpose. Krebs' Medium III (89), which was prepared by the following formula was used:-

0.9% (0.154M) NaCl	95
1.15% (0.154M) KCl	4
1.22% (0.11M) CaCl ₂	3
2.11% (0.154M) KH ₂ PO ₄	1
3.82% (0.154M) MgSO ₄ .7H ₂ O	1
1.3% NaHCO ₃	3
Na Phosphate Buffer	3
0.16M Na Pyruvate	4
0.1M Na Fumarate	7
0.16M Na-L-Glutamate	4
0.3M Glucose	5

Na Phosphate Buffer:

0.1M Na ₂ HPO ₄ (1.78% Na ₂ HPO ₄ .2H ₂ O) ..	100
0.1M NaH ₂ PO ₄ (1.38% NaH ₂ PO ₄ .H ₂ O) ...	25.
pH 7.4	

Since the metabolites present in KMI:II cannot be stored in solution longer than a week, they were freshly prepared each week. Concentrated stock solutions of salts were prepared and kept under refrigeration, being diluted before use and added to metabolites.

(b) Method

In order to determine the effect of the time factor in connection with the administration of each drug over the interval of the experiments, the animals were sacrificed at the rate of one weekly from each of the above groups, beginning one week after the start of injections.

The animals were weighed, and then killed by decapitation and placed in a cold ($2 - 5^{\circ} \text{C}$), moist chamber where the livers were quickly excised. Each liver was quickly weighed on a Gram-atic balance and then sliced using a Martin slicer (90). As the tissues were sliced they were placed upon tared pieces of waxed paper or tared fibre glass mats. These were kept in moist petri dishes in the refrigerated cabinet until all the sections were ready for weighing. After weighing, the tissues were immediately removed to the Warburg

flasks. Three samples were placed in KMIII and three in the Huston-Martin flasks (HM flasks). Both the standard flasks and the HM flasks were previously brought to a temperature of approximately 37° C by warming on a hot plate. The tissues spread out on the fibre glass mats were placed on the paddles in the HM flasks and those on waxed paper removed to the fluid medium. After attachment to the manometers, the flasks were placed in the constant temperature water bath at 37.9° C where oxygenation was carried out for two minutes. Ten minutes was allowed for thermal equilibration. The operation and weighings required from 20 to 25 minutes. Measurement of respiration was by the direct method of Warburg at a shaking rate of 120 cycles per minute.

For carbon dioxide absorption 0.2 ml of 10 per cent potassium hydroxide solution was placed in the center well of the standard flasks and was absorbed into filter paper discs placed on the bottom of the HM flasks. Each flask received 1.5 ml of solution, this being contained in the removable tray of the HM flasks. Readings were taken every 10 minutes for 90 minutes.

(c) Calculation of Results

Warburg manometers record changes in pressure of gas in the flask. The flask constant, coupled with the weight of the tissue is used to determine a standardized QO_2 . The QO_2 in this investigation expresses ml of oxygen consumed per gram of tissue (wet weight) per hour. Since the rate of respiration in artificial medium declines with time, the QO_2 value for zero time must be obtained by straight line extrapolation of the rates during the experimental period. The rate of respiration has been depressed by the cold during the operation procedure and returns to a maximum at the conclusion of thermal equilibrium. This rate of respiration is therefore the closest approximation to that in situ.

(d) Statistical Methods

A statistical analysis was conducted to determine the effect of drug administration upon the gross body weight and upon the liver weight relative to the body weight. The effect of the

drugs on the QO_2 values of the liver tissue was examined, and the mean QO_2 values were tested for significance using the Student's "t" test (91). In addition, a regression line was fitted to the QO_2 values obtained for each group and the regression coefficient b , which is the slope of the trend line of y upon x , was determined, where x represents the length of time of injection and y represents the QO_2 values. The value of b obtained was used to determine whether there was any correlation between the QO_2 values, the age of the animal, and the length of time of drug administration, by means of Student's "t" test. A modified Student's "t" test was used to determine the significance of the difference of the slopes of the regression lines for the QO_2 values of treated animals relative to the slope of the regression line for the QO_2 values of the normal animals. In each case the point of significance was determined at the 95 per cent level. Sample calculations showing the method of determination of the statistical significance of the data appear in the appendix.

(e) Histological Studies

Liver samples were taken from representative animals in each group, placed in 10 per cent formalin, and examined histologically. Slides were prepared using Hematoxylin and Eosin stain, and photomicrographs were taken at a magnification of 80 and 225 times.

VI. RESULTS

1. Gross Effects of Drug Treatment

The effects which the several treatments had on mortality, body weight, and liver weight are summarized in Tables I, II and III respectively.

The animals receiving carbon tetrachloride alone appeared wasted and sickly with extremely scruffy fur. Their peritoneal cavity was distended with ascitic fluid and they were difficult to handle, showing disorientation and hyperactivity. The liver was enlarged, fatty (whitish in color), fibrous and mushy, and difficult to slice. The average gross weight at the time of sacrifice was 329 grams, a value significantly below the normal of 381 grams, whereas the average liver weight, expressed as a percentage of the total body weight, was significantly above the normal.

In contrast, after carbon tetrachloride administration was stopped and nature was allowed to take its course, both the gross appearance and the condition of the liver appeared to return

toward the normal. The animals looked healthier and were easier to handle, although they retained their ascites. The livers, too, exhibited a more normal color, but still seemed fibrous upon slicing. The body weight increased to a point where it was no longer significantly different from the normal, while the liver weight was also within the normal range, probably due to a decrease in fat infiltration. Altogether the animals showed an amazing progress in the direction of normality, as is indicated by their mortality rate. While animals receiving carbon tetrachloride over the 15 weeks of the experiments had a 27 per cent mortality, those receiving carbon tetrachloride for only 4 weeks all lived for the entire time of the investigation.

Corticotropin-treated animals showed signs of lassitude and some toxicosis, although in other respects they appeared essentially normal. Gross body weights and liver weights were not significantly different from normal, but the mortality rate was 7 per cent of the animals treated.

Corticotropin and carbon tetrachloride administered together produced a deleterious effect upon the general condition of the animals as well

as upon the weight picture. The animals seemed small, somewhat sickly and hypoactive. Their gross body weight was significantly below normal in contrast to their liver weight which was greatly increased above normal. The livers appeared lighter in color than normal with signs of fat infiltration, although this phenomenon was less evident than when carbon tetrachloride was administered alone or when carbon tetrachloride and cortisone were given together. All these animals survived the course of injections.

When carbon tetrachloride administration was discontinued and then corticotropin given, the animals returned to a condition similar to those receiving corticotropin only. Both the body weight and the liver weight returned to normal, and all the animals survived the course of treatment.

Cortisone-treated animals, like the corticotropin-treated animals, were smaller than normal, although on the whole they appeared fairly healthy. However, in contrast to the corticotropin-treated animals, their livers were significantly enlarged above normal. Again in this group the mortality rate was high,

being 20 per cent of the animals treated.

The animals receiving cortisone along with carbon tetrachloride were obviously the most emaciated of the series. They suffered from diarrhoea, and continually appeared to be on the verge of death. They were the smallest animals throughout the series, and at the time of sacrifice weighed on an average only 211 grams. The liver was significantly enlarged above the normal and was mushy and very light in color. Twenty per cent of these animals died before termination of the experiments.

The animals receiving cortisone after carbon tetrachloride had been discontinued improved in appearance and gained weight. At the time of sacrifice the average weight was 270 grams, a figure still significantly below normal, but nevertheless an increase above the 211 grams of the previous group. The liver, too, was still enlarged and fibrous, but the color had returned almost to normal. However, these animals had a mortality rate of 30 per cent, which was the second highest in the series.

The first response of the animals receiving alcohol was manifested in diarrhoea, stupor, and

anorexia. Until approximately the fourth week the animals refused food and drink. However a tolerance seemed to develop, and although the animals remained smaller than normal, they appeared generally in good health, if somewhat sleepy. The liver also appeared normal.

Alcohol administered concurrently with carbon tetrachloride produced a general malaise which was manifested by a 40 per cent mortality. Here again the animals were small and wasted looking, with a body weight significantly below normal. Again the liver was significantly enlarged above normal, although fatty infiltration was at a minimum, comparable to that exhibited by animals receiving corticotropin and carbon tetrachloride.

Alcohol administered after the cessation of a series of carbon tetrachloride injections did not seem to enhance the previous injury. However, the animals still remained significantly below normal with regard to gross body weight and their livers remained significantly above normal with regard to relative weight. The general condition improved gradually, and the liver condition reverted toward the normal.

The healthiest appearance of all the drug-treated animals of the series was enjoyed by those receiving methionine. Their gross body weight was significantly depressed, but the relative liver weight was normal. Although the livers were light in color, the animals appeared frisky and generally in good health.

On the other hand, methionine proved to be a scourge in connection with fatty infiltration when administered along with carbon tetrachloride. Not only was the relative liver size the largest of the series, but the effect of methionine was evidenced by livers which were very soft and mushy, almost white in color, and very difficult to slice. The animals showed evidence of ascites, but otherwise appeared to be thriving.

Administration of methionine following carbon tetrachloride seemed to enhance the process of fat infiltration, although in other respects the animals showed a gradual return to normalcy.

TABLE I

THE EFFECT OF DRUGS ON THE MORTALITY RATE OF NORMAL
AND CARBON TETRACHLORIDE-TREATED RATS

	Per Cent Mortality
Normal	0
Carbon Tetrachloride	27
Carbon Tetrachloride discontinued	0
Corticotropin	7
Corticotropin and CCl ₄	0
Corticotropin after CCl ₄ discontinued	0
Cortisone	20
Cortisone and CCl ₄	20
Cortisone after CCl ₄ discontinued	30
Alcohol	0
Alcohol and CCl ₄	40
Alcohol after CCl ₄	10
Methionine	0
Methionine and CCl ₄	0
Methionine after CCl ₄ discontinued	0

TABLE II

THE EFFECT OF DRUGS ON THE GROSS BODY WEIGHT OF
NORMAL AND CARBON TETRACHLORIDE-TREATED RATS

	Average Weight	S.D.	Sig.
Normal	381	23.3	
CCl4	329	33.2	S
CCl4 discontinued	344	50.2	
Corticotropin	351	42.0	
Corticotropin and CCl4	294	31.4	S
Corticotropin after CCl4	356	54.5	
Alcohol	329	42.3	S
Alcohol and CCl4	271	26.1	S
Alcohol after CCl4	320	38.7	S
Methionine	348	30.4	S
Methionine and CCl4	310	47.6	S
Methionine after CCl4	347	48.4	S
Normal	330	36.2	
Cortisone	245	13.2	S
Cortisone and CCl4	211	35.4	S
Cortisone after CCl4	270	40.4	S
CCl4	273	35.2	S

Sig. means significantly different from normal.

TABLE III

THE EFFECT OF DRUGS ON THE RELATIVE LIVER WEIGHTS OF
NORMAL AND CARBON TETRACHLORIDE-TREATED RATS

	Liver Weight as Per Cent of Body Weight	S.D.	Sig.
Normal	3.35	0.12	
CCl ₄	4.43	0.48	S
CCl ₄ discontinued	3.57	0.32	
Corticotropin	3.14	0.93	
Corticotropin and CCl ₄	4.88	0.21	S
Corticotropin after CCl ₄	3.39	0.25	
Cortisone	4.49	0.24	S
Cortisone and CCl ₄	4.51	0.21	S
Cortisone after CCl ₄	4.27	0.45	S
Alcohol	3.22	0.89	
Alcohol and CCl ₄	4.68	0.16	S
Alcohol after CCl ₄	3.49	0.11	S
Methionine	3.45	0.34	
Methionine and CCl ₄	5.08	0.86	S
Methionine after CCl ₄	3.46	0.27	

Sig. means significantly different from normal.

2. Histological Effects of Treatment

Tables IV and V show the histological effects of administration of the various drugs and carbon tetrachloride over a period of 8 and 12 weeks respectively. Tables VI, VII and VIII summarize the individual histological effects of administration of the various drugs and carbon tetrachloride.

Although the results do not present a new concept concerning the toxicity of carbon tetrachloride to the liver, they show that damage does occur, and that corticotropin, cortisone, alcohol and methionine may play a part in ameliorating or accentuating such damage. This histological survey is included, therefore, with the aim of confirming the results obtained by tissue respiration.

Carbon tetrachloride administered alone produced a high incidence of lobular disarray, some centrilobular necrosis and minimal focal necrosis. Hydropic change and portal inflammatory infiltrate were evident, and binuclear cells were prominent. The beginning of a cirrhotic lesion was accompanied by fatty change and emphatic regenerative activity.

Carbon tetrachloride administered over a period of 4 weeks and then discontinued for another period of 4 weeks allowed the tissue to reconstitute itself. Portal inflammatory infiltrate and lobular disarray were present, although to a somewhat lesser degree than when the tissue was undergoing treatment with carbon tetrachloride. Regeneration also continued actively, but in other respects the tissue was essentially normal. A second period of 4 weeks without injections had no further ameliorating effect.

The 8 week series of cortisone injections had no effect upon the histological picture presented by the normal liver. However, cortisone administered for 8 weeks to animals concurrently receiving carbon tetrachloride produced some centrilobular necrosis, focal necrosis and lobular disarray. Hydropic change, portal inflammatory infiltrate and dispersed cytoplasmic basophilia were evident. Regenerative activity and fatty change were present, although the lesion had not progressed to cirrhosis.

When carbon tetrachloride was given for 4 weeks, discontinued, and followed by cortisone for 4 weeks, the livers improved and returned, with some exceptions, to a condition similar to the one shown when cortisone alone was administered

for the corresponding 8 week period. Binuclear cells, lobular disarray and regenerative activity remained evident.

Corticotropin, like cortisone, had no significant effect upon the normal liver. In contrast, administration of the hormone together with carbon tetrachloride for 8 weeks produced marked histological changes. The most prominent of these were extensive centrolobular necrosis, lobular disarray, hydropic change, and fat infiltration. In addition, binuclear cells, portal inflammatory infiltrate and regenerative activity were in evidence. A further 4 weeks of therapy advanced the lesion to the beginning cirrhotic state. Administration of corticotropin after carbon tetrachloride had been discontinued resulted in an improved condition similar to the one obtained when corticotropin was administered alone, with the exception of the continuance of binuclear cells and regenerative activity, indicating the presence of an altered cell population.

Alcohol administration left the liver essentially normal. Alcohol and carbon tetrachloride together, however, resulted in several interesting changes in the histological pattern. These

alterations were among the most conspicuous of any observed in all the groups comprising the series, and showed the most progression in the direction of a cirrhotic lesion. The most distinctive changes were marked portal inflammatory infiltrate, binuclear cells, and lobular disarray. Regeneration was proceeding very actively, and cirrhosis was present. Less marked, but still notable, was the presence of fatty change and central hydropic change, along with some centrolobular necrosis and focal necrosis.

Alcohol received by the animals following a series of carbon tetrachloride injections was well tolerated, and allowed the tissue to return almost to normal. Only the remaining binuclear cells and the active regeneration indicated that the cells had undergone carbon tetrachloride treatment.

Methionine administered for 8 weeks resulted in no significant changes in cellular architecture. However, after 12 weeks, a dispersal of cytoplasmic basophilia was noted. In contrast, methionine together with carbon tetrachloride produced centrolobular necrosis, focal necrosis, and extensive lobular disarray.

Binuclear cells were present and dispersion of cytoplasmic basophilic aggregates occurred. Fatty change and regenerative activity were the most distinctive of the whole series, while portal inflammatory infiltrate also was very noticeable. Cirrhosis was present. Altogether, the alterations in this group were comprehensive, comparable in toxicity to those obtained using alcohol and carbon tetrachloride, and exceeding those obtained using carbon tetrachloride alone.

When methionine was given for 4 weeks following a series of carbon tetrachloride injections, binuclear cells, lobular disarray, and regenerative activity remained prominent. In addition cirrhosis remained evident. A further 4 weeks of methionine had no appreciable effect in alleviating these alterations.

In summary, as shown by Tables VI, VII, and VIII, corticotropin, cortisone, alcohol and methionine had no effect on normal liver. Cortisone seemed to protect against the fatty change, formation of binuclear cells, lobular disarray and cirrhosis produced by carbon tetrachloride. Corticotropin, on the other hand, offered no protection and even accentuated the centrilobular

necrosis, the hydropic change, and the fatty infiltration of carbon tetrachloride. Alcohol, too, advanced the cirrhotic lesion. Portal inflammatory infiltrate, regeneration and cirrhosis were increased over the corresponding changes in the carbon tetrachloride group. Only fatty infiltration was decreased. Methionine accented only one feature of the carbon tetrachloride lesion, fatty infiltration.

Administration of carbon tetrachloride for 4 weeks, followed by a period of 8 weeks without injections, allowed the tissue to return in the direction of normalcy. The 4 drugs administered following the carbon tetrachloride had no appreciable effect on the histological picture except for methionine which produced extensive lobular disarray over and above that exhibited by the tissue attempting recovery from carbon tetrachloride without further treatment.

<p>Note: For each histological alteration the normal is 0, except for cytoplasmic basophilia where the normal is 4.</p>	Normal	1 0 4 0 0 0 0 0 0 0
	CCl4 discontinued	1 0 3-4 0 2 0 0 2 2 0
	ACTH	1 0 2-3 0 0 1 0 0 0 0
	ACTH and CCl4	1 2 0 2-3 0 3+ 2 2 1 0
	ACTH after CCl4	1+ 1 3-4 0 0 0 0 0 0 0
	Alcohol	1+ 0 3-4 0 0 0 0 0 0 0
	Alcohol after CCl4	1 0 3-4 0 0 1 0 0 1 2
	Methionine	1 0 3-4 0 1 0 0 1-2 2 0
	Methionine and CCl4	2 0 3-4 3 2 0 0 3 2-3 1
	Methionine after CCl4	1 0 4 0 2 2 0 0 0 0
	Portal Inflammatory Infiltrate	
	Hydropic Change (central)	
	Cytoplasmic Basophilia	
	Fatty Change	
	Binuclear Cells	
	Centrolobular Necrosis	
	Focal Necrosis	
	Lobular Disarray	
	Regenerative Activity	
	Cirrhosis	

THE HISTOLOGICAL EFFECTS OF DRUGS UPON THE LIVERS OF
NORMAL AND CARBON TETRACHLORIDE-TREATED RATS
(Drugs and Carbon Tetrachloride administered
for a period of 12 weeks)

TABLE V

TABLE VI

SUMMARY OF THE HISTOLOGICAL EFFECTS OF DRUGS ON
NORMAL RAT LIVER

	Normal	CCl ₄	Cortisone	Corticotropin	Alcohol	Methionine.
Portal Inflammatory Infiltrate	0	2+	0	0	0	0
Hydropic Change (central)	0	1+	0	0	0	0
Cytoplasmic Basophilia	4	3-4	3-4	4	4	4
Fatty Change	0	2+	0	0	0	0
Binuclear Cells	0	3	1	1	1	0
Centrolobular Necrosis	0	2+	0	0	0	0
Focal Necrosis	0	1+	0	0	0	0
Lobular disarray	0	3-4	0	1+	1	0
Regenerative Activity	0	2-3	0	1+	1	1
Cirrhosis	0	1+	0	0	0	0

Note: For each histological alteration the normal is 0, except for cytoplasmic basophilia where the normal is 4.

TABLE VII

SUMMARY OF THE HISTOLOGICAL EFFECTS OF DRUGS
UPON CARBON TETRACHLORIDE-TREATED RAT LIVER

	Normal	CCl ₄	Cortisone + CCl ₄	Corticotropin + CCl ₄	Alcohol + CCl ₄	Methionine + CCl ₄
Portal Inflammatory Infiltrate	0	2+	2	2	3	2+
Hydropic Change (central)	0	1+	1+	4	1	1
Cytoplasmic Basophilia	4	3-4	3+	3+	3-4	3
Fatty Change	0	2+	1	3-4	1	4
Binuclear Cells	0	3	1+	1-2	3+	3
Centrolobular Necrosis	0	2+	1+	4	1	2
Focal Necrosis	0	1+	1	1	1+	1+
Lobular disarray	0	3-4	1	3-4	4	4
Regenerative Activity	0	2-3	2-3	2	4	3+
Cirrhosis	0	1+	0	0	2	1+

Note: For each histological alteration the normal is 0, except for cytoplasmic basophilia where the normal is 4.

TABLE VIII

SUMMARY OF THE HISTOLOGICAL EFFECTS OF DRUGS ON
RAT LIVER AFTER A SERIES OF CARBON
TETRACHLORIDE INJECTIONS

	Normal	CCl4 discontinued	Cortisone after CCl4	Corticotropin after CCl4	Alcohol after CCl4	Methionine after CCl4
Portal Inflammatory Infiltrate	0	1+	1	1	1	1+
Hydropic Change (central)	0	0	0	0	0	0
Cytoplasmic Basophilia	4	4	4	4	4	4
Fatty Change	0	0	0	0	0	0
Binuclear Cells	0	2	1-2	1+	1+	2+
Centrolobular Necrosis	0	0	0	0	0	0
Focal Necrosis	0	0	0	0	0	0
Lobular Disarray	0	1+	1-2	1	1	3
Regenerative Activity	0	2-3	2	1-2	1-2	3
Cirrhosis	0	0	0	0	0	1

Note: For each histological alteration the normal is 0, except for cytoplasmic basophilia where the normal is 4.

3. Effects of Drug Treatment on Tissue Respiration

(Tables i - xxx appear in appendix)

1. Normal

Tables i and ii show the normal QO_2 values obtained for rat liver in KMIII and in HM flasks. The mean QO_2 values were 3.08 in KMIII and 3.14 in HM flasks. These values compare favorably with the findings of Huston and Martin (85). The regression lines calculated from the data of Tables i and ii had a positive slope of 0.82 in KMIII and 0.50 in the HM flasks, as is shown in Table XI. Both of these values were significantly different from zero at the 95 per cent level, indicating that the liver respiration of normal animals increased with the age of the animal over the time of the experiment.

2. Carbon Tetrachloride.

Tables iii and iv give the QO_2 values of rat liver after a series of carbon tetrachloride injections. The mean QO_2 values were 2.96 in KMIII and 3.09 in HM flasks. Comparable to the results obtained with normal animals, the mean

QO_2 value obtained in the KMIII flasks was somewhat lower than the mean QO_2 value obtained using the HM flasks. Carbon tetrachloride produced an initial depression of respiration below normal followed by a gradual rise until the QO_2 values were above normal, a phenomenon which was not indicated by taking a simple average of the QO_2 values, since the low values counterbalanced the high values, giving no indication of the progression which was taking place. The effect was shown to a greater extent in the HM flasks than in KMIII, a fact which was indicated by the slopes of the regression lines, which were 1.37 and 0.87 respectively. The slope of 1.37 obtained in the HM flasks was significantly different from zero, indicating that the respiration of the liver was directly dependent upon the length of time of administration of the carbon tetrachloride, being initially depressed, and gradually rising to a value above the normal QO_2 value. However, the QO_2 values were not significantly different from the normal in either KMIII or in HM flasks, as is indicated in Tables XI and XII, since the respiration of normal animals was directly dependent upon the age of the animal. Thus, carbon

tetrachloride had no significant effect upon the respiration of the liver.

3. Carbon Tetrachloride discontinued

When carbon tetrachloride was administered for 4 weeks, stopped, and the liver allowed to reconstitute itself, the mean QO_2 values obtained in KMIII and in HM flasks were 2.66 and 2.95 respectively. The mean value obtained in KMIII is significantly below the normal, although the one obtained in HM flasks is not. In addition, there was a wide difference in the slopes of the individual regression lines, the KMIII values having a negative slope of 0.79 and the HM flasks having values with a positive slope of 0.81. At the time when carbon tetrachloride injections were discontinued, the liver showed respiration in both flasks comparable to that shown in similar animals having undergone similar injections. However, from this point on, the respiration in the HM flasks increased and approached the normal respiration, while respiration in the KMIII flasks was depressed until it was significantly below the normal. The slopes of the regression lines

were not significantly different from zero; thus in both flasks the $\dot{Q}O_2$ values obtained were not dependent upon the time factor.

(Tables v and vi)

4. Corticotropin

Tables vii and viii show that corticotropin administered to normal rats caused depression of the liver respiration. The extent of depression, as indicated by the mean $\dot{Q}O_2$ values in Tables IX and X, was significantly below normal in KMIII but not in the HM flasks. The slopes of the regression lines displayed no significance from zero; thus the length of time of administration of the drug had no effect upon the $\dot{Q}O_2$ values. Also the slopes of the regression lines were not significantly different from those of the normal regression lines, indicating that the drug had no significant effect on the liver respiration. It is observed that here again the HM flasks gave a higher mean $\dot{Q}O_2$ than the KMIII flasks.

5. Corticotropin and Carbon Tetrachloride

Corticotropin administered simultaneously with carbon tetrachloride caused a reduction in liver respiration exceeding the reduction caused by either carbon tetrachloride or corticotropin administered singly. The mean QO_2 values, 2.46 and 2.92, observed in KMIII and in HM flasks respectively, were in both cases significantly below normal. The regression line for the QO_2 values in KMIII had a negative slope of 0.27 which was not significantly different from zero. The corresponding line for the HM flasks had a negative slope of 1.06 which also was not significant. Therefore the QO_2 values obtained were independent of the length of time of administration of the two drugs. However, the slope of the line for the QO_2 values in HM flasks was significantly different from the normal which indicates that corticotropin and carbon tetrachloride administered together have a depressant action upon liver respiration, which can be observed in HM flasks but not in KMIII. Similar to the results obtained above, the HM flasks again had a higher mean QO_2 than the KMIII flasks. (Tables ix and x)

6. Corticotropin after Carbon Tetrachloride
discontinued

Corticotropin administered after a series of carbon tetrachloride injections did not improve but rather further accentuated the initial depressive effect of carbon tetrachloride. Liver receiving comparable injections of the toxin and then left to recover had a respiration more nearly approaching normal than liver treated by injections of corticotropin subsequent to the carbon tetrachloride injections. Tables xi and xii give the QO_2 values for corticotropin administered after carbon tetrachloride, while Tables IX and X give a comparison of the mean QO_2 values, which for both flasks are significantly below the normal values. The length of time of administration of the drug had no effect on the respiration. The regression line for the KMIII values had a negative slope of 0.64, a value significantly below normal. In contrast, the regression line calculated from the QO_2 values for the HM flasks had a positive slope of 0.26, a value which was not significantly different from normal. Thus corticotropin may have a deleterious effect when administered following carbon tetrachloride, but this effect is more evident in KMIII than in HM flasks.

7. Cortisone

The effects produced upon liver respiration by cortisone therapy were variable, the KMIII flasks showing QO_2 values somewhat above normal, and the HM flasks showing QO_2 values below normal. The mean QO_2 values were 3.13 for KMIII and 2.94 for the HM flasks, the latter value being significantly below the normal. These results show the fallaciousness of merely calculating a simple average, since the slopes of the regression lines calculated from the data in Tables xiii and xiv show that in both cases cortisone initially increased and then gradually depressed the liver respiration to a level significantly below the normal. The effectiveness of the drug in causing this depression is independent of the time factor. Corticotropin produced depression to a deeper level than cortisone in KMIII, and to approximately the same level in HM flasks. However, the positive slopes of the corticotropin regression lines as compared to the negative slopes of the cortisone lines suggest that cortisone may have a more deleterious effect than corticotropin. These results were confirmed by the gross condition of the animals.

8. Cortisone and Carbon Tetrachloride

The effect of administering cortisone concurrently with carbon tetrachloride was not evidenced by examination of the mean $\dot{V}O_2$ values. Both in KMIII and HM flasks these values were not significantly different from the normal. Calculation of regression lines from the values shown in Tables xv and xvi indicated that time was not a factor in the effect of the drugs. Cortisone and carbon tetrachloride caused a depression which was significant in KMIII but not in HM flasks. The fact that a definite depression occurred, even this was not shown by the mean $\dot{V}O_2$ values, was evidenced by the negative slopes of the regression lines, which were 0.95 in KMIII and 0.92 in HM flasks. The net influence of cortisone and carbon tetrachloride upon liver respiration is comparable to the effect produced by corticotropin and carbon tetrachloride, although the former drugs had a much greater effect upon the gross condition of the animals than the latter ones.

9. Cortisone after Carbon Tetrachloride

Cortisone administered following a series of carbon tetrachloride injections produced an effect which was not shown by perusal of the mean QO_2 values. As indicated in Tables xvii and xviii, the mean QO_2 value for KMIII was 3.04, while for the HM flasks it was 2.89. Thus cortisone administered after carbon tetrachloride had no significant effect upon the mean QO_2 value in KMIII, since this value was approximately equivalent to those for the normal, carbon tetrachloride, and cortisone-treated animals. However, the mean QO_2 value in HM flasks was lower than the mean value for cortisone, cortisone and carbon tetrachloride together, carbon tetrachloride alone, carbon tetrachloride discontinued, and moreover, significantly lower than the normal value. Further examination of the data showed that the time variable and the QO_2 variable were independent. The regression lines were opposing in direction; the KMIII values had a positive slope of 0.61, while the HM flasks gave a negative slope of 0.34. These values were not significantly different from the slope of the normal line. Thus cortisone after carbon tetrachloride in both flasks caused a change in the trend of the depressive progression in the

direction of the normal. In KMIII the liver respiration improved over and above that shown by cortisone and carbon tetrachloride administered together, and above that shown by liver left alone after carbon tetrachloride treatment. In the HM flasks, cortisone therapy following carbon tetrachloride treatment resulted in improvement over the respiration shown when cortisone and carbon tetrachloride were administered together. However, although cortisone raised the depressed respiration initially, further injections did not improve the respiration as much as the improvement shown when similar livers were merely left to reconstitute themselves naturally without further therapeutic treatment. Thus cortisone administered after carbon tetrachloride may or may not play a role in alleviating the carbon tetrachloride lesion. Nevertheless the effect of cortisone following carbon tetrachloride was a considerable improvement over the effect of corticotropin in similar circumstances.

10. Alcohol

The effect of alcohol was not clearly shown by the mean QO_2 values. In KMIII the mean QO_2 was significantly below the normal, while in the HM flasks it was significantly above the normal. (Tables xix and xx) However, the slope of the regression line in KMIII was 0.53 in a positive direction, whereas the slope pertaining to the comparable HM QO_2 values was practically negligible. (Tables XI and XII) In both cases the slopes of the regression lines were not significantly different from normal, indicating that alcohol had no significant effect upon normal liver respiration. Administration of the drug over an extended period of time had no effect upon the respiration.

11. Alcohol and Carbon Tetrachloride

Interesting results were obtained following the concurrent administration of alcohol and

carbon tetrachloride. In both flasks the mean QO_2 values were depressed to a level significantly below the normal. (Tables IX and X) Regression lines calculated from the data of Tables xxi and xxii indicated that time was not a factor in the resulting liver respirations. In KMIII the regression coefficient had a negative value of 1.06; in HM flasks it had a negative value of 0.23. The latter value was significantly different from the normal. Alcohol and carbon tetrachloride administered simultaneously had a depressant action upon liver respiration, a fact which is more evident in HM flasks than in KMIII. The respiratory depression caused by these two drugs descended to a level below that caused by cortisone or corticotropin when administered concurrently with carbon tetrachloride, and far below that caused by carbon tetrachloride alone.

12. Alcohol after Carbon Tetrachloride

Alcohol administration following carbon tetrachloride administration was well tolerated. (Tables xxiii and xxiv) In KMIII flasks, the mean QO_2 value of 2.75 remained significantly below the normal, but in HM flasks, the value of 3.23 was

essentially normal. The slopes of the regression lines for the data were positive, being 0.72 for KMIII and 0.75 for the HM flasks. Thus alcohol administered after carbon tetrachloride, in contrast to alcohol administered concurrently with carbon tetrachloride, had an ameliorating effect upon the liver lesion, bringing the respiration to within the normal range both in KMIII and in the HM flasks. Furthermore, examination of the data indicated in HM flasks that the ameliorating effect was dependent upon the length of time the drug was administered. The tissue recovered in HM flasks at a rate comparable to that of liver tissue receiving no drug therapy following carbon tetrachloride; in KMIII recovery was at a rate in excess of that shown by damaged liver receiving no supportive therapy.

13. Methionine

In KMIII the mean QO_2 value was 2.71; in the HM flasks it was 3.07. (Tables xxv and xxvi) Thus, although the HM mean value was nearly normal, the KMIII mean value was significantly depressed

below normal. The regression line for KMIII had a positive slope of 0.55, and the regression line for the HM values had a negative slope of 0.05. Both slopes were not significantly different from normal indicating that although the action of methionine bordered on the level of depression, the action was not significant. However, in KMIII, the action of the drug varied with the length of time of administration.

14. Methionine and Carbon Tetrachloride

The mean QO_2 values, as shown in Tables xxvii and xxviii, indicated that methionine and carbon tetrachloride administered together depressed the respiration of the liver significantly. The mean QO_2 value obtained from the KMIII flasks was the lowest of the whole series. The slopes of the regression lines were 0.01 in KMIII and 2.37 in HM flasks. The latter value was significantly different from the normal, indicating that methionine and carbon tetrachloride given together significantly depressed the liver respiration in HM flasks. In addition, in these flasks, the drugs were found to exert an effect directly proportional to the length of time of

administration. In the KMIII flasks, the net effect was one of depression. However, in the HM flasks, the tissue seemed to recover from an initial deep depression and the QO_2 values rose until they exceeded the normal values.

15. Methionine after Carbon Tetrachloride discontinued

Methionine given after the administration of carbon tetrachloride did not improve the hydrocarbon lesion, but rather kept the respiration on a level of depression similar to that shown by methionine and carbon tetrachloride administered together. (tables xxix and xxx) The mean QO_2 values were 2.50 in KMIII and 2.59 in HM flasks, both of which were significantly depressed below the normal. The positive slope of 0.13 in KMIII and the negative slope of 0.43 in HM flasks were not significantly different from the normal, thus indicating that methionine probably had no effect with regard to aiding in the reconstitution of the liver, but on the other hand did not interfere with the regeneration process which proceeded following the carbon tetrachloride stimulus.

TABLE IX

SUMMARY OF MEAN $\dot{V}O_2$ VALUES OF NORMAL, CARBON
TETRACHLORIDE AND DRUG TREATED RATS
MEASURED IN KMIII

	No. Of Expts.	Mean	s.d.m.	Sig.
Normal	62	3.08	0.52	
CCl ₄	42	2.96	0.48	
CCl ₄ discontinued	30	2.66	0.45	S
Corticotropin	40	2.66	0.34	S
Corticotropin + CCl ₄	29	2.46	0.58	S
Corticotropin after CCl ₄ discontinued	29	2.55	0.42	S
Cortisone	30	3.13	0.50	
Cortisone + CCl ₄	30	3.07	0.52	
Cortisone after CCl ₄ discontinued	27	3.04	0.44	
Alcohol	44	2.80	0.56	S
Alcohol + CCl ₄	18	2.53	0.61	S
Alcohol after CCl ₄ discontinued	26	2.75	0.60	S
Methionine	40	2.71	0.33	S
Methionine + CCl ₄	24	2.25	0.51	S
Methionine after CCl ₄ discontinued	25	2.50	0.53	S

s.d.m. - standard deviation of the mean

Sig. - a significant difference from the normal.

TABLE X

SUMMARY OF MEAN QO_2 VALUES OF THE LIVERS OF
NORMAL, CARBON TETRACHLORIDE AND DRUG
TREATED RATS MEASURED IN HM FLASKS

	No. of Expts.	Mean	s.d.m.	sig.
Normal	57	3.14	0.40	
CCl ₄	44	3.09	0.70	
CCl ₄ discontinued	29	2.95	0.64	
Corticotropin	40	2.96	0.52	
Corticotropin + CCl ₄	27	2.92	0.58	S
Corticotropin after CCl ₄ discontinued	28	2.62	0.78	S
Cortisone	29	2.94	0.40	S
Cortisone + CCl ₄	30	3.06	0.67	
Cortisone after CCl ₄ discontinued	26	2.89	0.42	S
Alcohol	41	3.34	0.47	S
Alcohol + CCl ₄	18	2.50	0.65	S
Alcohol after CCl ₄ discontinued	23	3.23	0.62	
Methionine	42	3.07	0.60	
Methionine + CCl ₄	25	2.79	0.75	S
Methionine after CCl ₄ discontinued	30	2.59	0.66	S

s.d.m. - standard deviation of the mean

sig. - a significant difference from the normal.

TABLE XI

THE EFFECT OF TIME AND DRUGS UPON TISSUE
RESPIRATION OF THE LIVER
MEASURED IN KMIII

- A. The effect of time upon the respiration of the livers of normal, carbon tetrachloride and drug-treated animals.
- B. The effect of drugs upon the respiration of the livers of normal and carbon tetrachloride-treated animals.

	s_y	s_x	s_{xy}	b	r	Sig. A.	B.
Normal	0.52	0.25	5.28	p 0.82	0.40	S	
CCl4	0.48	0.15	1.99	p 0.87	0.28		
CCl4 discon.	0.45	0.20	3.41n	n 0.79	0.36		S
ACTH	0.33	0.24	1.68	p 0.28	0.21		
ACTH + CCl4	0.58	0.21	1.18n	n 0.27	0.10		
ACTH after CCl4	0.42	0.19	0.64n	n 0.16	0.08		S
Cortisone	0.50	0.17	2.65n	n 0.96	0.32		S
Cortisone + CCl4	0.52	0.12	1.42n	n 0.95	0.22		S
Cortisone after CCl4	0.44	0.12	0.94	p 0.61	0.17		
Alcohol	0.56	0.27	3.78	p 0.53	0.25		
Alcohol + CCl4	0.61	0.14	2.07n	n 1.06	0.27		
Alcohol after CCl4	0.60	0.20	2.89	p 0.72	0.24		
Methionine	0.33	0.27	3.92	p 0.55	0.44	S	
Meth. + CCl4	0.51	0.20	0.02	p 0.01	0.00		
Methionine after CCl4	0.53	0.22	0.64	p 0.13	0.05		

*Footnote on the following page.

TABLE XI (continued)

- s_x - The standard deviation of the mean values of x , where x represents the time in days.
- s_y - The standard deviation of the mean values of y , where y represents the QO_2 values.
- s_{xy} - The covariance of the pairs of values x, y .
- b - The regression coefficient is the slope of the trend line of y upon x .
- r - Pearson's correlation coefficient. It indicates the degree of association between the QO_2 variable and the time variable.
- Sig. A. - The significance of the regression coefficient. Indicates that the value of the QO_2 variable is dependent upon the time variable.
- Sig. B. - The significance of the slope of the regression lines of the carbon tetrachloride and drug treated animals as compared to the slope of the regression line calculated for normal animals.
- n - indicates a negative slope of the regression line.
- p - indicates a positive slope of the regression line.
- S - significance calculated at the 95 per cent level.

TABLE XII

THE EFFECT OF TIME AND DRUGS UPON TISSUE
RESPIRATION OF THE LIVER MEASURED IN
HM FLASKS

- A. The effect of time upon the respiration of the livers of normal, carbon tetrachloride and drug-treated animals.
- B. The effect of drugs upon the respiration of the livers of normal and carbon tetrachloride-treated animals.

	s_y	s_x	s_{xy}	b	r	Sig.	
						A.	B.
Normal	0.40	0.25	3.12	p 0.50	0.31	S	
CCl ₄	0.70	0.16	3.44	p 1.37	0.31	S	
CCl ₄ discon.	0.64	0.20	3.53	p 0.81	0.26		
ACTH	0.52	0.25	4.18	p 0.66	0.32		
ACTH + CCl ₄	0.58	0.21	4.60n	n 1.06	0.38		S
ACTH after CCl ₄	0.78	0.19	0.92	p 0.26	0.06		
Cortisone	0.39	0.16	1.48n	n 0.56	0.24		S
Cortisone + CCl ₄	0.67	0.12	1.38n	n 0.92	0.17		
Cortisone after CCl ₄	0.42	0.12	0.55n	n 0.34	0.10		
Alcohol	0.47	0.26	0.45n	n 0.07	0.04		
Alcohol + CCl ₄	0.65	0.14	0.46n	n 0.23	0.05		S
Alcohol after CCl ₄	0.62	0.21	3.20	p 0.75	0.79	S	
Methionine	0.60	0.27	0.35n	n 0.05	0.02		
Meth. + CCl ₄	0.75	0.20	9.79	p 2.37	0.64	S	S
Methionine after CCl ₄	0.66	0.22	2.11n	n 0.43	0.14		

* Footnote on the following page.

TABLE XII (continued)

- s_x - The standard deviation of the mean values of x , where x represents the time in days.
- s_y - The standard deviation of the mean values of y , where y represents the QO_2 values.
- S_{xy} - The covariance of the pairs of values x, y .
- b - The regression coefficient is the slope of the trend line of y upon x .
- r - Pearson's correlation coefficient. It indicates the degree of association between the QO_2 variable and the time variable.
- Sig. A. - The significance of the regression coefficient. Indicates that the value of the QO_2 variable is dependent upon the time variable.
- Sig. B. - The significance of the slope of the regression lines of the carbon tetrachloride and drug treated animals as compared to the slope of the regression line calculated for the normal animals.
- n - indicates a negative slope of the regression line.
- p - indicates a positive slope of the regression line.
- s - significance calculated at the 95 per cent level.

Table XIII gives a summary of the significant effects of the various drugs upon the respiration of normal and carbon tetrachloride-treated rats. Figures I to XVI give a graphical representation of these effects.

A. Mean QO_2

The mean QO_2 values for KMIII were significantly below normal in the following cases;

- (a) when carbon tetrachloride was discontinued and the liver was allowed to follow its own pathway toward reconstitution;
- (b) when corticotropin, corticotropin and carbon tetrachloride, and corticotropin after carbon tetrachloride were administered;
- (c) when alcohol, alcohol and carbon tetrachloride, and alcohol after carbon tetrachloride were administered; and
- (d) when methionine, methionine and carbon tetrachloride, and methionine after carbon tetrachloride were administered.

The mean QO_2 values were significantly below normal in HM flasks in the following cases;

- (a) when corticotropin was given concurrently with carbon tetrachloride, and when corticotropin was given following carbon tetrachloride;
- (b) when cortisone alone was injected, and when cortisone was administered after carbon tetrachloride;
- (c) when alcohol and carbon tetrachloride were administered concurrently; and
- (d) when methionine, methionine and carbon tetrachloride, and methionine after carbon tetrachloride were administered.

The respiration was stimulated to give a mean QO_2 value significantly above normal when alcohol was given and respiration was measured in HM flasks.

B. Effect of time upon liver respiration

- (a) The liver respiration of normal animals was found to be directly dependent upon the age of the animal both in KMIII and in HM flasks.
- (b) The QO_2 values obtained when animals were treated for an extended period with carbon tetrachloride proved to be directly dependent

upon the length of time of administration of the drug, the initial depressive state being followed by a gradual rise to a level above the normal CO_2 values. This effect was evident in both flasks, but was significant at the 95 per cent level only in the HM flasks.

- (c) Alcohol administered after a series of carbon tetrachloride injections produced an improved effect upon the respiration which was directly dependent upon the length of time of administration of the alcohol. The improvement was significant however only in the HM flasks.
- (d) Methionine given to rats with otherwise normal livers affected the rate of respiration, depending upon the length of time of its administration. In this instance the effect was significant in KMIII and not in HM flasks.
- (e) The respiratory rate varied with time when methionine and carbon tetrachloride were administered simultaneously, but again the effect was significant in HM flasks but not in KMIII.

C. The effect of the various drugs on the QO_2 of normal and carbon tetrachloride-treated animals

- (a) Carbon tetrachloride resulted in an initial depression of liver respiration which was gradually overcome by the regenerating liver until the QO_2 values reached and finally exceeded the level of the normally respiring livers. The respiration was not depressed or stimulated in either KMIII or HM flasks to the level of significance, however.
- (b) When carbon tetrachloride was given for a period of 4 weeks and then discontinued, the respiration rates in the KMIII and in the HM flasks were equivocal, the former falling until they were significantly different from the normal, and the latter rising until they were equivalent to the normal.
- (c) Corticotropin caused respiratory depression which was not significant in either KMIII or in HM flasks. Corticotropin injected concurrently with carbon tetrachloride produced depression of the liver respiration to a plane significantly below normal in HM flasks but not

in KMIII. Corticotropin given following carbon tetrachloride allowed the liver to return to a respiratory rate which was normal in HM flasks, but significantly below normal in KMIII.

- (d) Cortisone, in contrast, was in itself instrumental in significantly depressing the liver respiration, both in KMIII and in HM flasks. In addition, cortisone given simultaneously with carbon tetrachloride significantly depressed liver respiration in KMIII although not in HM flasks. However, cortisone administered following carbon tetrachloride produced an equivocal result in that rather than further depressing the carbon tetrachloride lesion, it allowed the respiratory rate to return to a level not significantly different from normal.
- (e) Alcohol administered alone, with carbon tetrachloride, and after carbon tetrachloride, showed no significant effect in KMIII. However, in HM flasks, alcohol administered along with carbon tetrachloride caused a significant depression of respiration. Alcohol administered after a series of carbon tetrachloride injections permitted the liver respiration to return to normal.

- (f) Methionine showed no significant effect in KIII whether administered along with carbon tetrachloride, after carbon tetrachloride, or alone. In HM flasks, methionine and carbon tetrachloride given together caused a significant depression in liver respiration. When carbon tetrachloride was administered, discontinued, and then methionine administered, the liver respiration returned to within the normal range.

TABLE XIII

SUMMARY OF THE EFFECTS OF DRUGS ON THE
RESPIRATION OF THE LIVER OF NORMAL
AND CARBON TETRACHLORIDE-TREATED RATS

	KMIII			HM FLASKS		
	Sig A	Sig B	Sig C	Sig A	Sig B	Sig C
Normal		S			S	
CCl ₄					S	
CCl ₄ discontinued	S		S			
ACTH	S					
ACTH + CCl ₄	S			S		S
ACTH after CCl ₄	S		S	S		
Cortisone			S	S		S
Cortisone + CCl ₄			S			
Cortisone after CCl ₄				S		
Alcohol	S			S		
Alcohol + CCl ₄	S			S		S
Alcohol after CCl ₄	S				S	
Methionine	S	S				
Methionine + CCl ₄	S			S	S	S
Methionine after CCl ₄	S			S		

S - Significance calculated at the 95 per cent level.

Sig. A. - The significance of the mean $\dot{Q}O_2$ values of carbon tetrachloride and drug treated rats as compared to the mean $\dot{Q}O_2$ of normal rats.

Sig. B. - The significance of the regression coefficient. Indicates that the value of the $\dot{Q}O_2$ variable is dependent upon the time variable.

Sig. C. - The significance of the slope of the regression lines of carbon tetrachloride and drug treated animals as compared to the slope of the regression line calculated for normal animals.

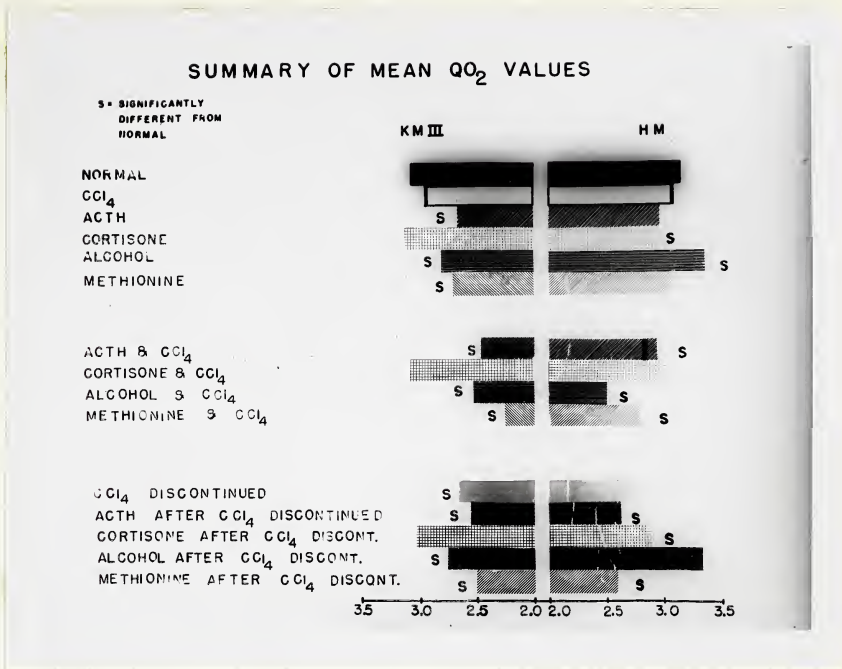


FIGURE I

THE EFFECTS OF DRUGS ON THE MEAN $\dot{Q}O_2$ VALUES OF
NORMAL AND CARBON TETRACHLORIDE-TREATED RATS

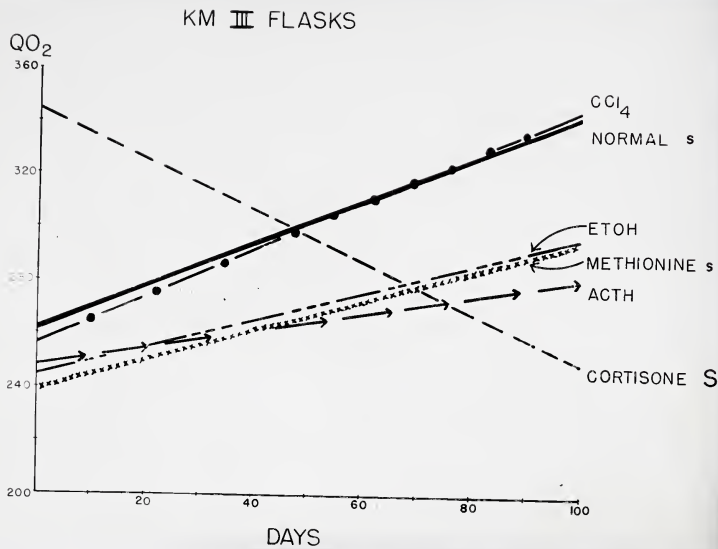


FIGURE II

THE EFFECTS OF DRUGS ON THE LIVER RESPIRATION
OF NORMAL RATS IN KMIII

s - The significance of the regression coefficient.
Indicates that the value of the QO_2 variable
is dependent upon the time variable.

S - The significance of the slope of the regression lines
of carbon tetrachloride and drug treated animals as
compared to the slope of the regression line
calculated for normal animals.

* QO_2 values x 100

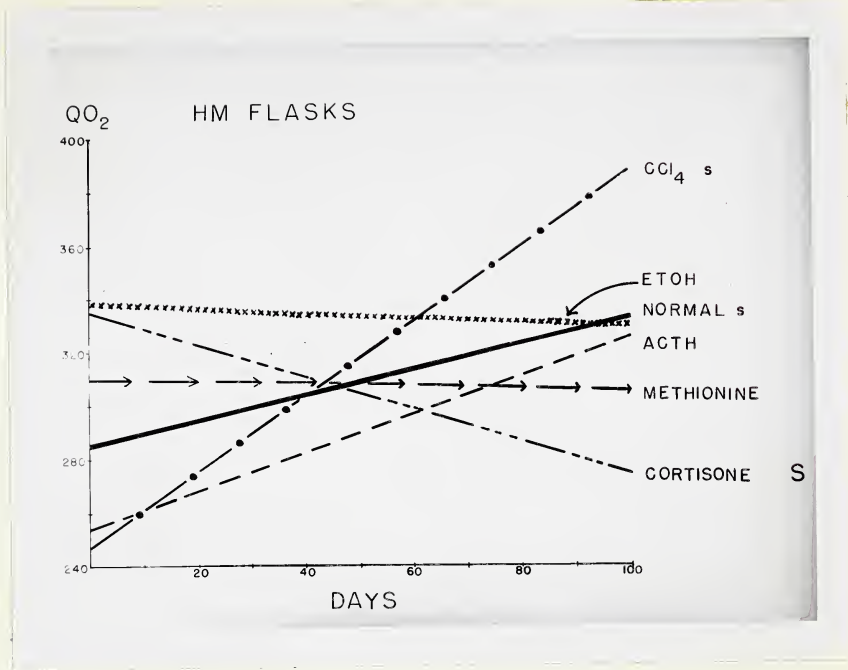


FIGURE III

THE EFFECTS OF DRUGS ON THE LIVER RESPIRATION
OF NORMAL RATS IN HM FLASKS

- s - The significance of the regression coefficient. Indicates that the value of the QO₂ variable is dependent upon the time variable.
- S - The significance of the slope of the regression lines of carbon tetrachloride and drug treated animals as compared to the slope of the regression line calculated for normal animals.

* QO₂ values x 100

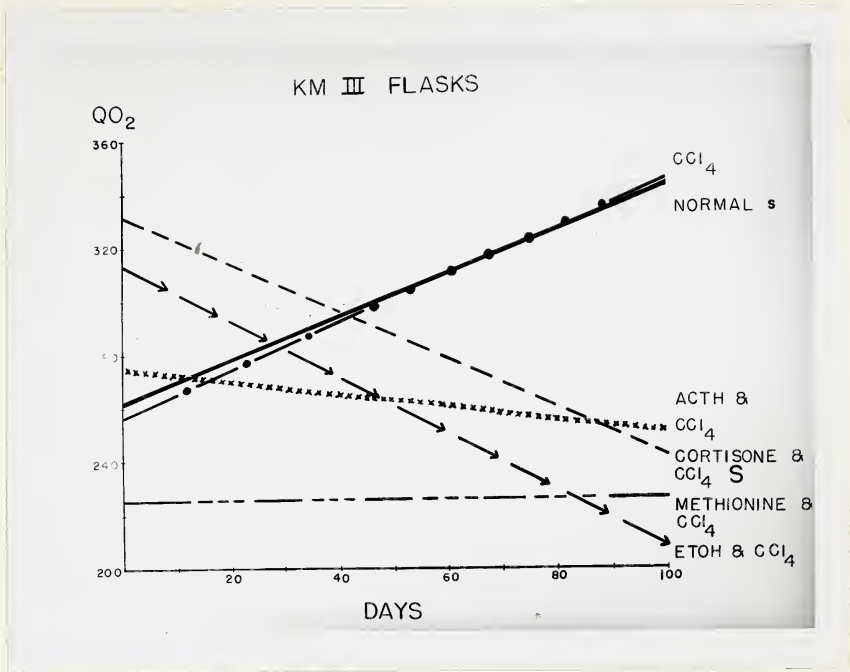


FIGURE IV

THE EFFECTS OF DRUGS ON THE LIVER RESPIRATION
OF CARBON TETRACHLORIDE TREATED RATS IN
KMIII

s - The significance of the regression coefficient.
Indicates that the value of the QO₂ variable is
dependent upon the time variable.

S - The significance of the slope of the regression
lines of carbon tetrachloride and drug treated
animals as compared to the slope of the regression
line calculated for normal animals.

* QO₂ values x 100

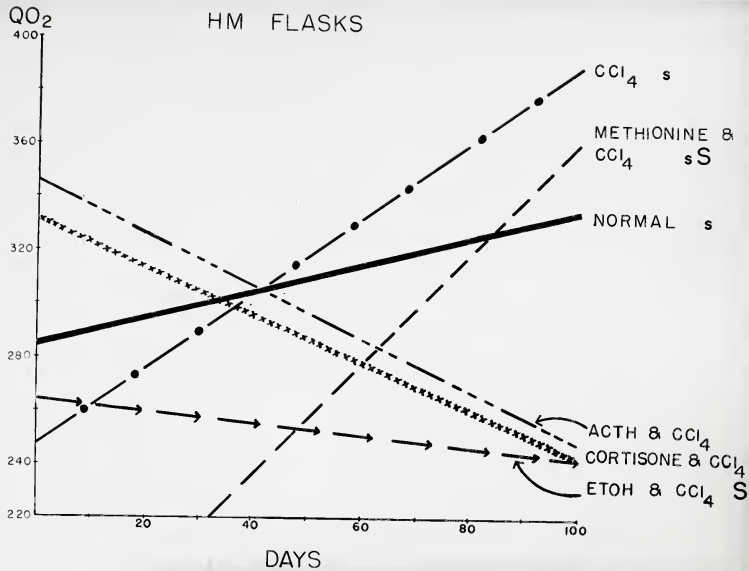


FIGURE V

THE EFFECTS OF DRUGS ON THE LIVER RESPIRATION
OF CARBON TETRACHLORIDE TREATED
RATS MEASURED IN HM FLASKS

s - The significance of the regression coefficient.
Indicates that the value of the QO_2 variable
is dependent upon the time variable.

S - The significance of the slope of the regression
lines of carbon tetrachloride and drug treated
rats as compared to the slope of the regression
line calculated for normal rats.

* QO_2 values x 100

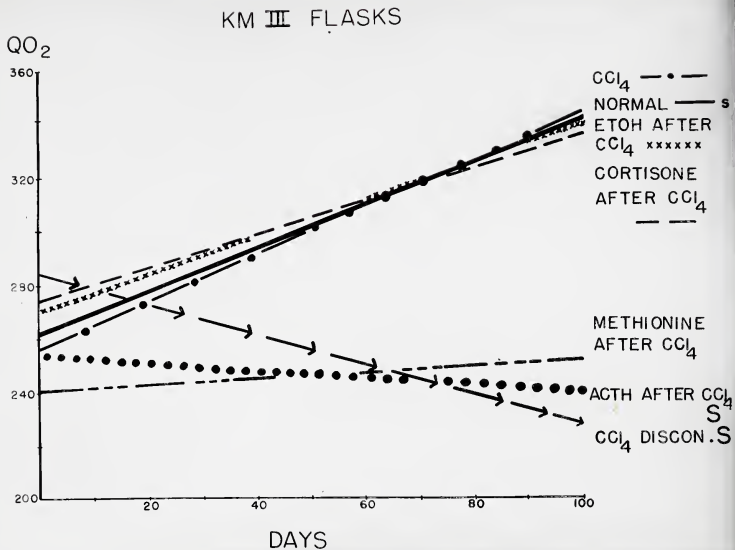


FIGURE VI

THE EFFECTS OF DRUGS ON THE LIVER RESPIRATION
OF RATS AFTER CARBON TETRACHLORIDE
DISCONTINUED, MEASURED IN KMIII

s - The significance of the regression coefficient. Indicates that the value of the QO_2 variable is dependent upon the time variable.

S - The significance of the slope of the regression lines of carbon tetrachloride and drug treated rats as compared to the slope of the regression line calculated for normal rats.

* QO_2 values x 100

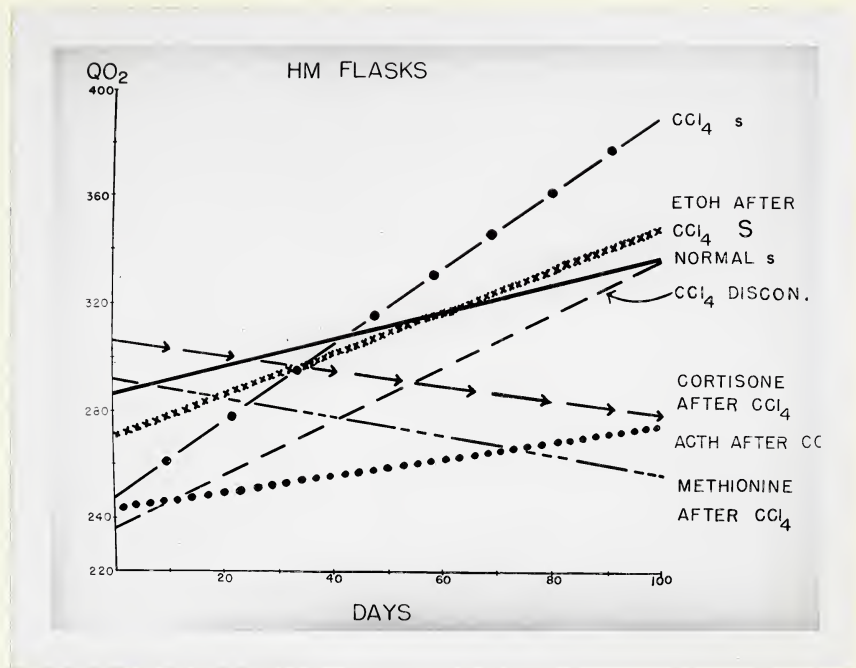


FIGURE VII

THE EFFECTS OF DRUGS ON THE LIVER RESPIRATION
OF RATS AFTER CARBON TETRACHLORIDE DISCONTINUED,
MEASURED IN HM FLASKS

s - The significance of the regression coefficient.
Indicates that the value of the QO_2 variable
is dependent upon the time variable.

S - The significance of the slope of the regression
lines of carbon tetrachloride and drug treated
rats as compared to the slope of the regression
line calculated for normal rats.

* QO_2 values x 100

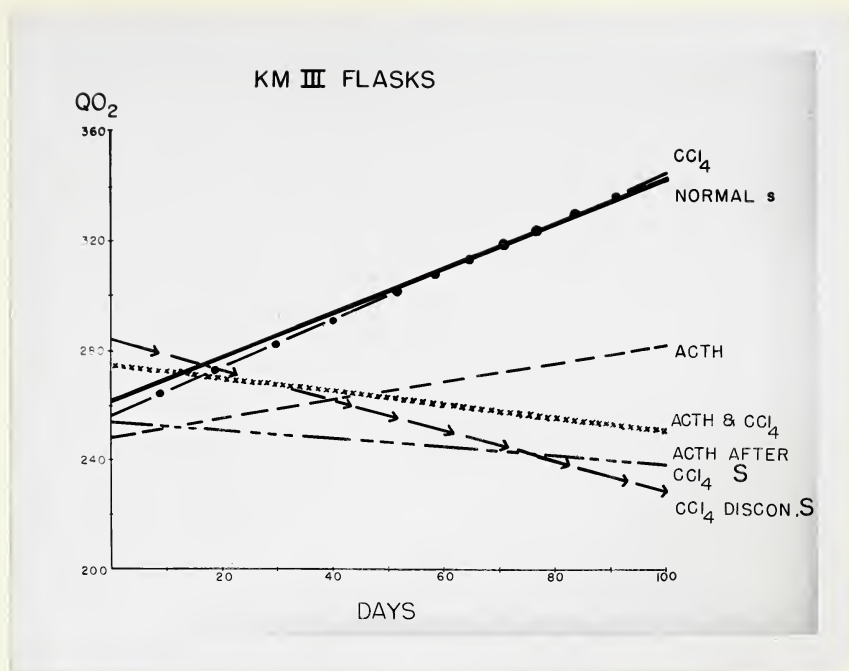


FIGURE VIII

THE EFFECT OF CORTICOTROPIN ON THE LIVER RESPIRATION
OF NORMAL AND CARBON TETRACHLORIDE TREATED RATS,
MEASURED IN KMIII

- s - The significance of the regression coefficient. Indicates that the value of the QO_2 variable is dependent upon the time variable.
- S - The significance of the slope of the regression lines of carbon tetrachloride and drug treated rats as compared to the slope of the regression line calculated for normal rats.

* QO_2 values x 100

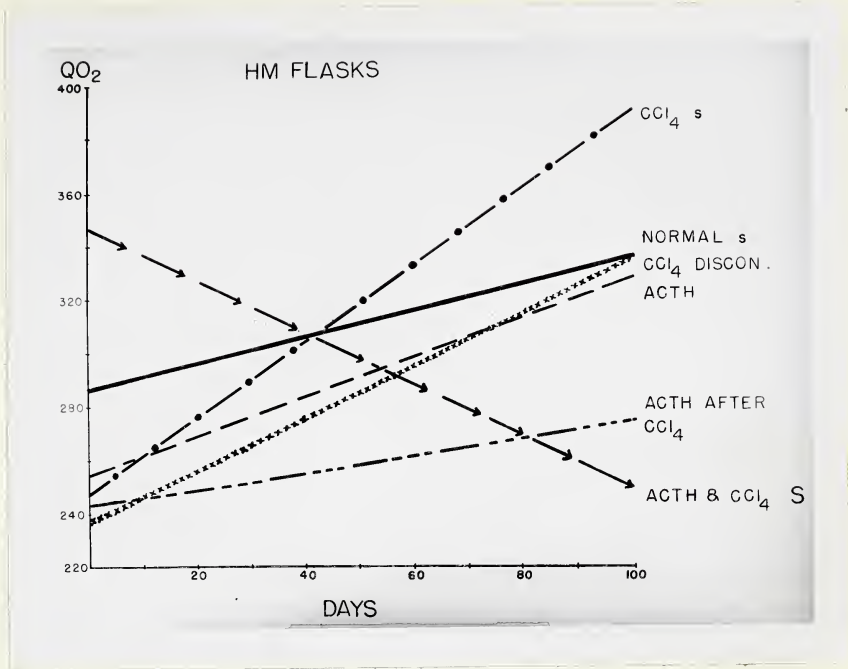


FIGURE IX

THE EFFECT OF CORTICOTROPIN ON THE LIVER RESPIRATION
OF NORMAL AND CARBON TETRACHLORIDE-TREATED RATS,
MEASURED IN HM FLASKS

s - The significance of the regression coefficient.
Indicates that the value of the QO_2 variable
is dependent upon the time variable.

S - The significance of the slope of the regression
lines of carbon tetrachloride and drug treated
rats as compared to the slope of the regression
line calculated for normal rats.

* QO_2 values x 100

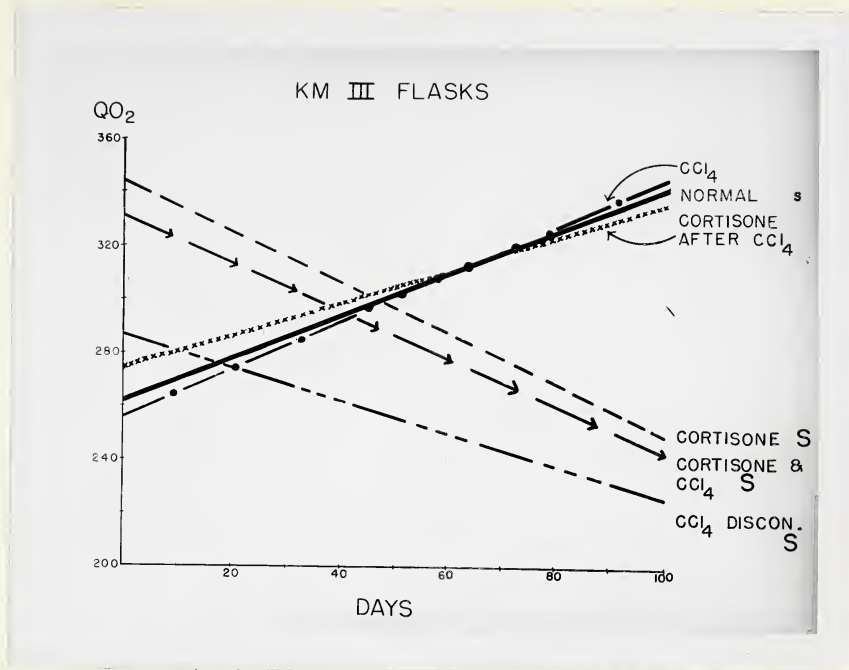


FIGURE X

THE EFFECT OF CORTISONE ON THE LIVER RESPIRATION
OF NORMAL AND CARBON TETRACHLORIDE
TREATED RATS, MEASURED IN KMIII

- s - The significance of the regression coefficient. Indicates that the value of the QO_2 variable is dependent upon the time variable.
- S - The significance of the slope of the regression lines of carbon tetrachloride and drug treated rats as compared to the slope of the regression line calculated for normal rats.

* QO_2 values x 100

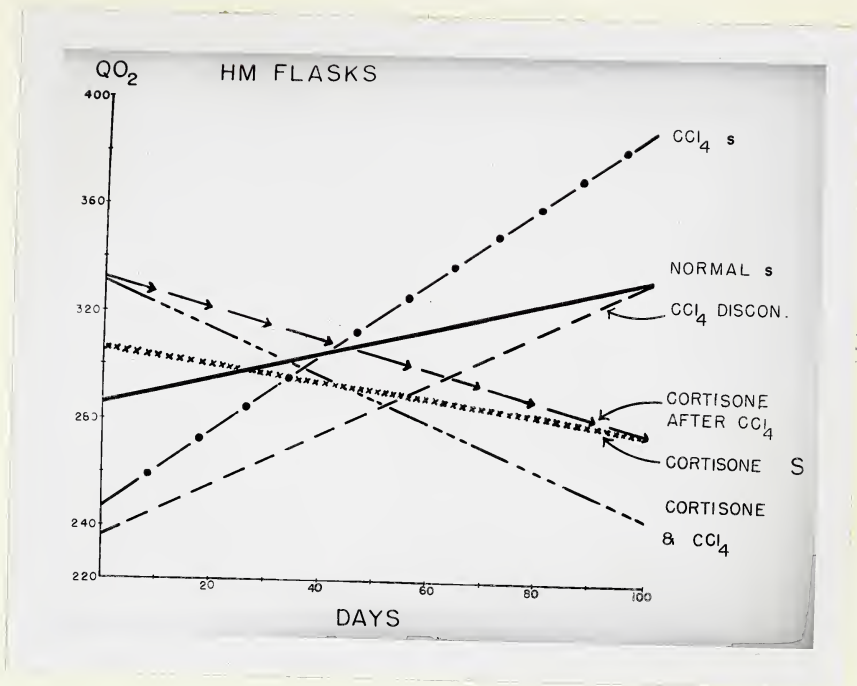


FIGURE XI

THE EFFECT OF CORTISONE ON THE LIVER RESPIRATION
OF NORMAL AND CARBON TETRACHLORIDE TREATED
RATS, MEASURED IN HM FLASKS

s - The significance of the regression coefficient.
Indicates that the value of the QO₂ variable
is dependent upon the time variable.

S - The significance of the slope of the regression
lines of carbon tetrachloride and drug treated
rats as compared to the slope of the regression
line calculated for normal rats.

* QO₂ values x 100

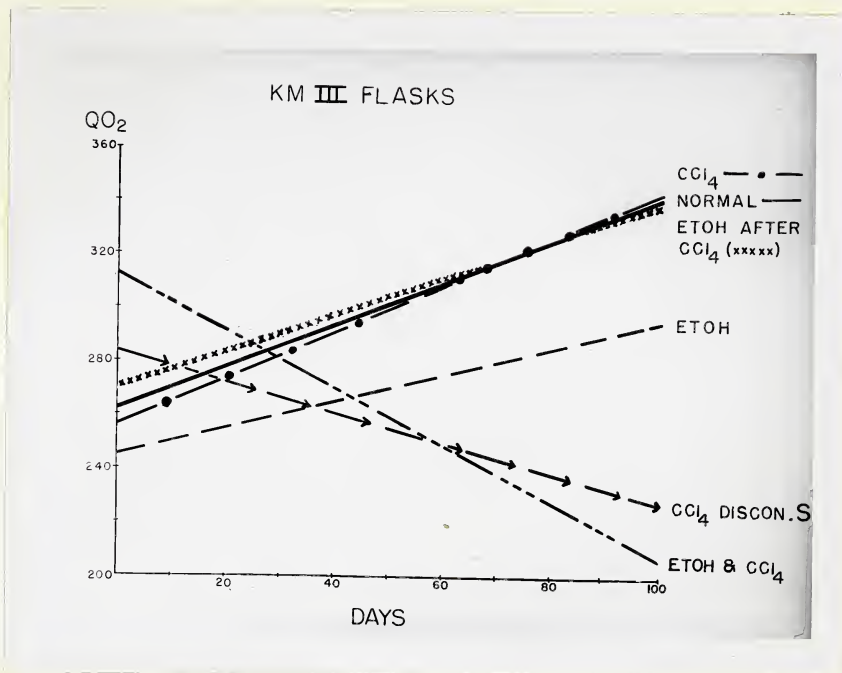


FIGURE XII

THE EFFECT OF ALCOHOL ON THE LIVER RESPIRATION
OF NORMAL AND CARBON TETRACHLORIDE-TREATED
RATS, MEASURED IN KMIII

s - The significance of the regression coefficient.
Indicates that the value of the QO_2 variable
is dependent upon the time variable.

S - The significance of the slope of the regression
lines of carbon tetrachloride and drug treated
rats as compared to the slope of the regression
line calculated for normal rats.

* QO_2 values x 100

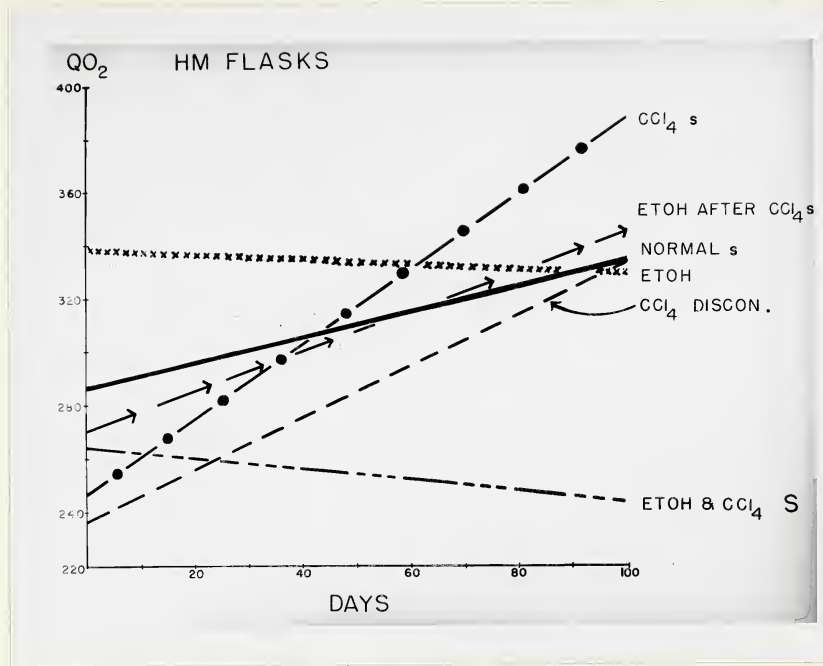


FIGURE XIII

THE EFFECT OF ALCOHOL ON THE LIVER RESPIRATION
OF NORMAL AND CARBON TETRACHLORIDE-TREATED
RATS, MEASURED IN HM FLASKS

- s - The significance of the regression coefficient.
Indicates that the value of the QO_2 variable
is dependent upon the time variable.
- S - The significance of the slope of the regression
lines of carbon tetrachloride and drug treated
rats as compared to the slope of the regression
line calculated for normal rats.

* QO_2 values x 100

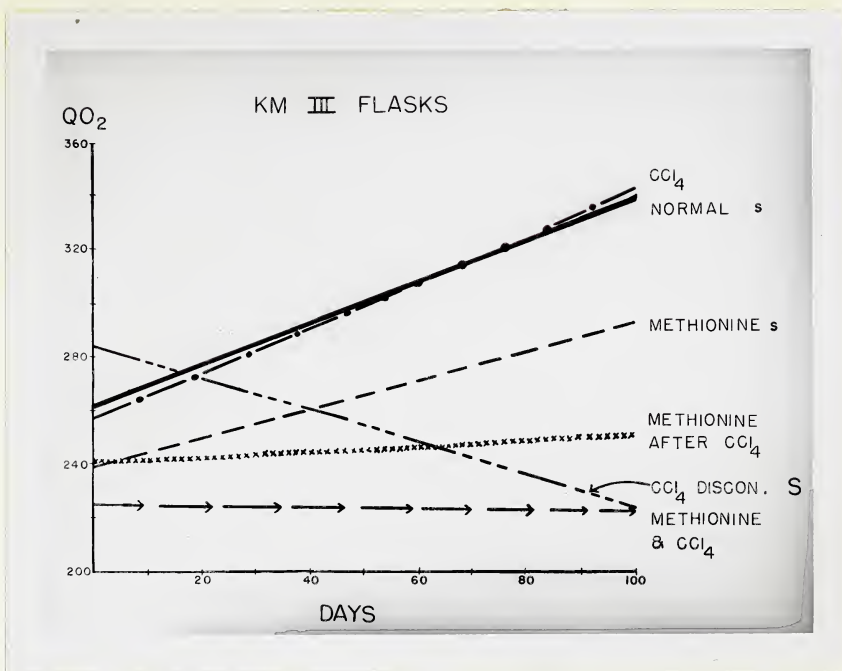


FIGURE XIV

THE EFFECT OF METHIONINE ON THE LIVER RESPIRATION
OF NORMAL AND CARBON TETRACHLORIDE-TREATED
RATS, MEASURED IN KMIII

s - The significance of the regression coefficient.
Indicates that the value of the QO_2 variable
is dependent upon the time variable.

S - The significance of the slope of the regression
lines of carbon tetrachloride and drug treated
rats as compared to the slope of the regression
line calculated for normal rats.

* QO_2 values x 100

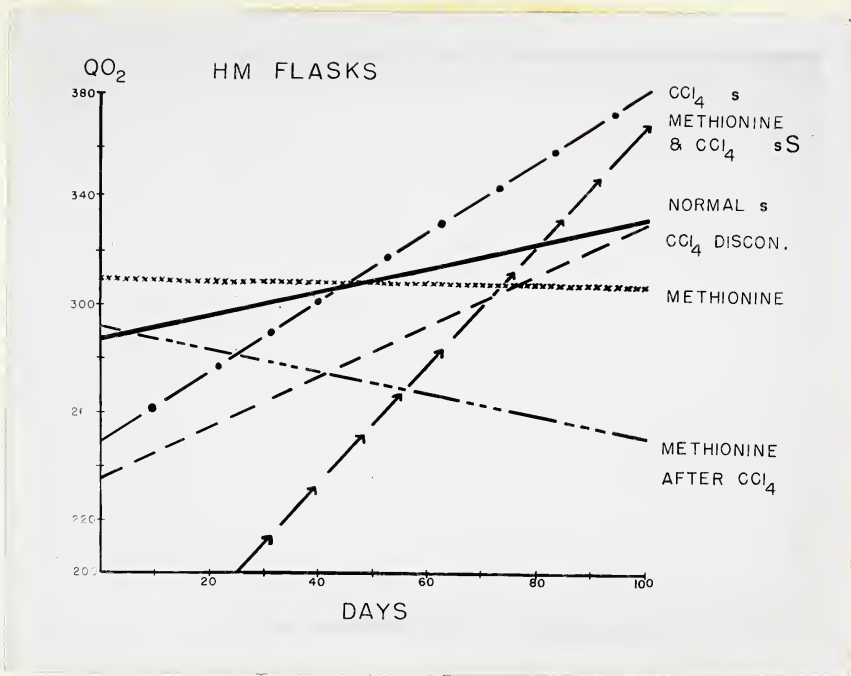


FIGURE XV

THE EFFECT OF METHIONINE ON THE LIVER RESPIRATION
OF NORMAL AND CARBON TETRACHLORIDE
TREATED RATS, MEASURED IN HM FLASKS

s - The significance of the regression coefficient.
Indicates that the value of the QO_2 variable
is dependent upon the time variable.

S - The significance of the slope of the regression
lines of carbon tetrachloride and drug treated
rats as compared to the slope of the regression
line calculated for normal rats.

* QO_2 values x 100

VII. DISCUSSION

(a) General Discussion

A discussion of these results must be prefaced by some comments on the procedure proposed by Huston and Martin (84,85) for the determination of tissue respiration in contact with oxygen. The in-vitro evaluation of the pharmacological action of drugs at the cellular level after administration of the drug in-vivo is complicated in standard Warburg methods by such factors as modification of the drugs and tissue metabolites by the liquid suspension medium. Cellular effects demonstrated by the addition of drugs in-vitro may or may not represent the response in the intact animal, particularly in view of possible differential tissue distribution and sensitivity of the drug. Huston and Martin showed that tissue respiration can be measured with the tissues suspended in a gaseous phase of oxygen on fibre glass mats. This technique avoids variations due to different liquid media and permits quantitative assessment in-vitro of the tissue effects of the drugs administered to the intact animal.

Rodnight and McIlwain (92) compared rates of respiration of brain, kidney, diaphragm and liver without added media and in olive oil, light paraffin and silicone fluid. In each case respiration rates were initially higher than those observed in saline which were run at the same time. They found that unless glucose was added the rate of respiration fell quite rapidly in brain.

Drabkin and Marsh (93) using a moist chamber respirometer on a principle similar to that of the Huston-Martin technique found that they could incubate tissue slices for as long as ten hours without appreciable diminution of the rates of oxygen uptake. They found that tissues remained viable two to three times longer than in conventional Warburg technique.

Some advantages to the technique of administering the drug to the animal and examining the tissues in oxygen would appear to be:-

- (a) the drug has been administered in-vivo and the distribution and response has been governed by the intact animal; adding the drug from the side arm in-vitro presents a completely artificial situation;
- (b) the drug has not been diluted or extracted from the tissues by a liquid medium;
- (c) variable influences due to ions or metabolites in the medium are avoided.

Disadvantages or limitations of the procedure may be summarized as follows:-

- (a) once the tissue is placed on the mat further drugs and metabolites cannot be added to it. The technique therefore does not lend itself to an examination of substrate phenomena;
- (b) the tissue cannot act as its own control as is the case when the drug is added from a side arm. It is necessary to run control series of non-treated animals;
- (c) a not too serious disadvantage and one which is inherent in all tissue respiration studies is that once the tissue is removed from the body it progressively departs from physiological normalcy. Many factors are involved, not all of which are known. Some of the more obvious factors are loss of hormone and nervous control, limitation of supply of metabolites and ions and accumulation of metabolic end products. However, since the primary interest is the effect of the drug on the tissue at the time it is removed from the body, that is the in-vivo effect, this disadvantage is not serious.
- (d) a possible disadvantage is that the tissue in contact with oxygen and not supplied with nutrient may burn itself up. This situation would be indicated by a more rapid fall in slope of the graph

and at the end of an hour the respiration rate might be expected to be below that of the tissues in fluid. However it was found that the tissues on the mats had the least diminution of oxygen consumption.

The above disadvantages are minimized by the procedure of extrapolation to zero time. Rate of respiration has been reduced in the cold during the preliminary manipulations and returns to a maximum at the conclusion of equilibration which is the point of extrapolation. This figure, so obtained, would appear to most closely approximate the in-vivo situation.

The administration of carbon tetrachloride and other drugs to the intact animal is the only procedure which could be used in a study of this kind, since the action of carbon tetrachloride on the liver depends upon the size of dose, the frequency of administration, and the number of doses, factors which depend upon the in-vivo method of administration. The disadvantages of using the regular Warburg technique have been discussed, and it would appear that the technique of Huston and Martin would be a useful method for investigating the effects of various drugs on liver respiration of normal and carbon tetrachloride-treated rats.

Previous work dealing with the hepatotoxic effects of carbon tetrachloride has been concerned with the histological effects of the drug (8 - 25). Calvert and Brody (26), who reported on the respiration effect of carbon tetrachloride on various liver enzymes used an in-vitro technique which may or may not represent the in-vivo activities.

Much investigation has been carried out concerning the effects of various drugs on the normal and carbon tetrachloride-treated animal liver. However the work has been done either histologically, by means of tissue respiration in-vitro, or by means of various other biochemical methods. For example, the action of cortisone on normal heart and diaphragm respiration has been measured in-vivo, but the action on other normal tissues has been measured only by the in-vitro technique. Furthermore, the action of cortisone with regard to carbon tetrachloride-induced damage of the liver has been qualified only by means of histological examination. C₁₄-labelled glucose and fructose have been used to measure the action of cortisone on liver glucose metabolism. The effects of methionine on liver metabolism have been qualified by means of histological techniques and various other methods, including measurement of

the nitrogen balance and liver function tests. In addition, much clinical investigation has been carried out concerning the effects of various drugs in liver disease. However, whether or not the experimental picture can be correlated with the clinical picture remains to be exemplified.

b) The Effects of the Drugs on Normal and Carbon Tetrachloride-Treated Animals.

1. Normal Animals

Throughout the 15 week period during which the liver respiration of the animals was determined, the normal animals gained weight from 120 grams to an average of 381 grams at the time of sacrifice. This final weight was the highest of the entire series, thus indicating that in all cases, treatment with the various drugs and carbon tetrachloride interfered with the normal growth of the animals. The liver respiration of the normal animals was shown to be a variable directly dependent upon the age of the animal. The mean QO_2 values obtained for normal animals in this work were found to be slightly higher,

but not significantly different from those reported by Huston and Martin (85). The increase above the value reported by these workers may be accounted for by the fact that their animals were sacrificed at an average weight of approximately 250 grams.

2. Carbon Tetrachloride

Oxygen uptake measurements indicated that the liver respiration was initially depressed by carbon tetrachloride, but that the liver was able to overcome this depression with the result that respiration returned to normal, or in the case of the HM flasks, to a value above normal. Statistically, at the 95 per cent level, the respiration was essentially normal. The histological picture confirmed these results. The carbon tetrachloride treated livers showed evidence of a beginning cirrhotic lesion accompanied by fatty change and energetic mitotic activity. Thus the liver appeared to be undergoing an active regenerative process which probably accounts for the apparently normal respiration, since it was proven statistically that for HM flasks the QO_2 values were directly dependent

upon the length of time of carbon tetrachloride administration. A similar phenomenon was observed in KMIII, but the data were not significant at the 95 per cent level.

These results appear to agree with data reported by Islami et al. (18) who found that injury to the liver produced a stimulus for regeneration which was so potent that rats with advanced cirrhosis could recovery completely using the residual liver after 70 per cent hepatectomy. In this group the rats had livers which were significantly enlarged above normal, which further indicated that liver cell proliferation had taken place at an increased rate, accompanied consequently by a respiration rate which increased above the initial level of depression. These results are not substantiated by those of Calvert and Brody (26) who found depression of liver respiration as a result of carbon tetrachloride administration. However these workers did not administer the toxin over an extended period of time; hence they did not measure the respiration of a rapidly regenerating liver. Glynn and Himsworth (23) suggested that swelling of the hepatic cells as a result of carbon tetrachloride in the circulation produced an ischemia in the organ which brought about a deficiency in the oxygen supply of the

centrolobular cells resulting in necrosis. However, Daniel et al (24) questioned the involvement of anoxia in carbon tetrachloride-induced necrosis based on the lack of evidence of impaired circulation of blood in the liver after carbon tetrachloride administration. The results obtained in this work, rather than reflecting a state of anoxia within the cell, would seem to agree with results obtained by Christie and Judah (22) who proposed that carbon tetrachloride acts directly on the mitochondria to produce a disorganization of the topographical relationships of the enzymes and a consequent disruption of metabolic activity.

3. Carbon Tetrachloride Discontinued

When carbon tetrachloride was administered for 4 weeks, discontinued, and the liver allowed to reconstitute itself, the gross appearance of the animals returned to normalcy. The histological work showed that regeneration was actively continuing, and although some lobular disarray was still present, the liver had returned almost to a normal condition. These findings confirmed the results obtained in HM flasks, but not those

obtained in KMIII. The regression lines calculated from the CO_2 values indicated that respiration returned to within the normal range in the HM flasks, but was depressed to a level significantly below normal in KMIII. The results obtained in HM flasks agree with investigations carried out by Cameron and Karunaratne (16) who reported that following cessation of regular carbon tetrachloride injections over short intervals, rat liver showed a gradual recovery from early monolobular cirrhosis.

4. Corticotropin

Measurements of oxygen uptake indicated that corticotropin had a slight depressant action on liver respiration which was however non-significant in both KMIII and HM flasks. Substantiation of these results was obtained by examination of the gross animal weights and the relative liver weights, which were within the normal range. In addition, the histological picture presented by these livers was normal. The apparent lassitude and toxicosis experienced by these animals was not reflected by their liver condition, although it was shown in their mortality rate which was 7 per cent of the animals treated.

5. Corticotropin and Carbon Tetrachloride

These drugs administered concurrently had a depressant action upon liver respiration which was significant in HM flasks but not in KMIII. Histological examination confirmed these results, showing extensive cellular changes which advanced to the beginning cirrhotic state. In addition, the livers were significantly enlarged above normal while the gross body weight was significantly below normal. Williams and Flink (70) tried corticotropin on patients with chronic liver disease and were doubtful of any beneficial effect, which would seem to agree with the results obtained in this study.

6. Corticotropin after Carbon Tetrachloride.

Administration of corticotropin after carbon tetrachloride had been discontinued resulted in an improved condition histologically, similar to the one obtained when corticotropin was administered alone, with the exception of the continuance of an altered cell population and regenerative activity.

In addition, the animal weights and the relative liver weights returned to normal, and all the animals survived. However, in contrast, the QO_2 values showed a further depression below the initial depressive level produced by the action of carbon tetrachloride. Livers receiving no treatment following carbon tetrachloride injections showed a higher respiratory rate than livers receiving corticotropin therapy. This effect was significant in KMIII but not in HM flasks. Wahi et al (71,72) noted that carbon tetrachloride caused functional damage to the adrenals and in addition may be toxic to the pituitary. Wool and Goldstein (77) reported that the anterior pituitary plays a role in fat mobilization in connection with the consequent production of a fatty liver. To complicate the situation, Brown et al (68) in their clinical studies noted reduction in fibrosis and fat infiltration after corticotropin. However the mechanism by which corticotropin exerts its effect on metabolism is unknown, so that at the present time, workers have merely speculated with regard to the effect of corticotropin on liver damaged by treatment with carbon tetrachloride.

7. Cortisone

In both KMIII and in HM flasks, cortisone depressed the liver respiration to a level significantly below the normal. Corticotropin produced depression to a deeper level than cortisone in KMIII, and to approximately the same level in HM flasks. However, the positive slopes of the corticotropin regression lines as compared to the negative slopes of the cortisone lines suggest that cortisone may have a more deleterious effect on normal liver than corticotropin. The livers of these animals were significantly enlarged above normal, and the mortality rate was 20 per cent of the animals treated. However the histological picture was not different from the normal. Various authors (78,79) reported that the steroids depress the metabolism of the tissues to which they are added by in-vitro methods. However, using in-vivo techniques, Lacroix and Leusen (83) found that cortisone has an action on tissue metabolism which includes a sex and tissue-linked specificity. Thus cortisone in the experiments of this study may exert an effect on metabolism which is peculiar to the liver.

1. Cortisone and Carbon Tetrachloride

Cortisone and carbon tetrachloride administered together caused depression of liver respiration to the level of significance in KMIII but not in HM flasks. The condition of the liver was also reflected in the general condition of the animals. Diarrhoea, emaciation, and fatty, enlarged livers led to a 20 per cent mortality rate. Moreover the histological picture was one of necrosis, distorted lobular architecture, fat infiltration, and regeneration. These factors indicated that cortisone exerted a deleterious effect upon the liver lesion produced by carbon tetrachloride.

Many authors have presented conflicting reports concerning the effects of cortisone in similar circumstances (16,17,18,28,29). Cortisone has been reported to reduce the fibrous content of the liver, decrease inflammation, decrease fat infiltration, and improve plasma protein concentration. On the contrary, it has also been reported to aggravate the cirrhotic lesion, producing deterioration of liver function, and interfering with the activity of various enzyme systems. Wahi et al (71,72) noted that carbon tetrachloride alters the ability of the liver cells to metabolize steroid hormones which results in a disproportionate

accumulation of these substances in the body. Such an accumulation would affect the anterior pituitary and hamper its capacity to elaborate corticotropin. Thus cortisone and carbon tetrachloride administered together may not only reduce the functional capacity of the liver, but also reduce the organism's inherent ability to cope with its environment by abolishment of the stress response.

9. Cortisone after Carbon Tetrachloride

When cortisone was administered after carbon tetrachloride, the general appearance of the animals improved as did the histological appearance of the liver. The liver was still significantly enlarged and fibrous, with binuclear cells, lobular disarray and regenerative activity, but the general appearance showed improvement. However the mortality rate was an inexplicably high 40 per cent. The respiration also showed a trend to return in the direction of the normal. In KMIII the respiration improved above the level of cortisone and carbon tetrachloride administered together, as well as above the level shown by liver left without treatment after receiving carbon tetrachloride. In the HM flasks, respiration was improved over that evidenced when cortisone and carbon tetrachloride

were administered together, but not as much as that shown by livers left to reconstitute themselves after carbon tetrachloride administration. Thus cortisone probably plays only a minor role, if any, in reconstitution of the liver recovering from a carbon tetrachloride-induced lesion.

10. Alcohol

Alcohol had no apparent effect upon the respiration nor upon the histological picture shown by the normal liver. The general condition of the animals was also good, although they suffered from diarrhoea and anorexia which resulted in weight loss which kept them smaller than their normal counterparts throughout the time of the experiments.

11. Alcohol and Carbon Tetrachloride

Alcohol and carbon tetrachloride administered together had a depressant action upon the liver which exceeded that produced by cortisone or corticotropin administered concurrently with carbon tetrachloride, and greatly exceeded that caused by carbon tetrachloride alone. Distinctive also

were the histological changes produced by alcohol and carbon tetrachloride. The cirrhotic lesion was the most marked of any group in the series. These animals experienced a mortality rate of 40 per cent, the highest of the series. They appeared sickly and emaciated, and their livers were significantly enlarged. It has been suggested that alcohol increases the absorption and thus the toxicity of carbon tetrachloride, because the hydrocarbon is soluble in alcohol. Moreover, the high caloric content of alcohol may increase the need for lipotropic substances and thus lead to pathological changes in the liver despite an otherwise adequate diet. Carbon tetrachloride can be oxidized to phosgene which can condense in-vitro to form ethylchloroformate. Guild et al (60) speculated that possibly this sequence of events can occur in-vivo. In any event, regardless of the mechanism of action, alcohol greatly increased the toxicity of carbon tetrachloride as shown by the various measurements undertaken in these experiments.

12. Alcohol after Carbon Tetrachloride

Surprisingly enough, alcohol administered following carbon tetrachloride was well tolerated by the animals. The animals remained significantly small and their livers were significantly large, but their general condition improved gradually until they appeared normal. Histologically, only binuclear cells and active regeneration indicated that the cells had undergone treatment with carbon tetrachloride. Examination of the QO_2 values indicated that alcohol administered following carbon tetrachloride allowed the respiration to return to within the normal range. In HM flasks the liver recovered at a rate comparable to that of liver tissue receiving no drug therapy following treatment with carbon tetrachloride; in KMIII recovery was at a rate in excess of that shown by damaged liver receiving no supportive therapy after carbon tetrachloride. Thus alcohol administered following carbon tetrachloride may have had an ameliorating effect upon the liver lesion. In any event, it did not interfere with normal recovery of the tissue.

13. Methionine

The action of methionine upon liver respiration bordered on the level of depression, but was not significant either in KMIII or in HM flasks. Methionine administered for 8 weeks showed no effect on cellular activity. However, after 12 weeks, dispersion of cytoplasmic basophilia was noted. This effect may correlate with the fact that the QO_2 values of methionine-treated animals depended upon the length of time of administration of the drug. The general appearance of the methionine-treated animals was the best of all the animals in the series. They appeared thriving and frisky, and although their gross weights were significantly low, their liver weights were normal.

14. Methionine and Carbon Tetrachloride

Methionine and carbon tetrachloride administered together caused depression of liver respiration. This effect was not significantly below normal in KMIII. In HM flasks the tissue apparently recovered from an initial deep depression and the QO_2 values rose until they exceeded the normal

values. The slope of the regression line was significantly different from the normal, and in addition, the effect of the drugs was shown to be dependent upon the length of time of administration. The general appearance of the animals was good, although their livers were very light, soft, and enlarged, showing extensive fatty infiltration. The histological alterations in these livers were also extensive, comparable in toxicity to those obtained using alcohol concurrently with carbon tetrachloride. The fatty change and regenerative activity seen were the most pronounced of the series. These results would appear to agree with observations by Munro and Mukerji (55) who found that addition of excessive amounts of individual amino acids to the diets of experimental animals gave rise to amino acid imbalances and toxicities. Several authors have made contradictory observations which indicate that methionine exerts a favorable effect in prevention of experimental injury in animals. Goodman and Gilman (2) proposed that methionine can protect against the enhanced susceptibility to liver damage which occurs in protein deficient states, but has no beneficial action after the organ has been injured.

Similarly Cohen et al (53) found that once cirrhosis is fully established, the addition of methionine did not bring about repair or regeneration of the liver.

15. Methionine after Carbon Tetrachloride

Administration of methionine following carbon tetrachloride seemed to enhance fatty infiltration of the liver, but in other respects the animals showed a gradual return to normalcy. However methionine produced no ameliorating effect in the histological pattern. Binuclear cells, lobular disarray, regenerative activity and cirrhosis remained evident. The QO_2 values obtained indicated that methionine had no effect with regard to aiding in the reconstitution process of the liver. Thus methionine would appear to have no value as a lipotropic agent in the treatment of carbon tetrachloride injury to the liver.

(c) Discussion of the Huston-Martin Technique

In addition to examining the effects of various drugs on the respiration of normal and carbon tetrachloride-treated animals, it was the aim of this research to further examine the Huston-Martin technique as a tool for pharmacologic investigations.

The CO_2 values obtained in HM flasks were comparable to those obtained in KMIII, a solution rich in substrate. In some cases a greater sensitivity was noted in KMIII; in others the HM flasks appeared more sensitive. However, the overall pattern of the effects of the drugs was equivalent, indicating that the technique of determining tissue respiration in oxygen is a useful tool for pharmacologic studies.

In order to study the effect of carbon tetrachloride in producing a cirrhotic lesion of the liver, it is obvious that the drug cannot be tipped in from the side arm, but must be administered to the animal for an extended period prior to the tissue respiration studies. Moreover, the measurement of the effect of various drugs

upon this lesion also involves administering the drug to the intact animal prior to the respiration studies. Thus it is felt that the Huston-Martin technique is well adapted to this type of research problem.

The findings of this work would seem to indicate that the Huston-Martin technique has several advantages over the conventional Warburg technique in pharmacologic studies. Any inadequacies of the Huston-Martin method are largely those inherent in any procedure where the tissues are removed from the animal and studied in-vitro.

VIII. SUMMARY AND CONCLUSIONS

The aim of this research was to determine the effects of various drugs upon the liver respiration of normal and carbon tetrachloride-treated animals, and to confirm these effects by means of gross examination of the animals and by means of a histological survey.

Carbon tetrachloride, corticotropin and cortisone were administered by subcutaneous injection. Methionine and alcohol were administered in the drinking water. Carbon tetrachloride was administered alone and concurrently with each of the 4 drugs. In addition carbon tetrachloride was given for 4 weeks followed by a period of rest for an additional 8 weeks. To determine the effect of the four drugs upon the recovery of the liver, each was administered following a series of carbon tetrachloride injections. To determine whether the drugs had any effect on normal liver, each was administered alone to normal animals over the time of the experiments.

The tissues were removed and the oxygen consumption measured in Krebs Medium III (KMIII) and in oxygen (HM flasks). The animal weights

and the respective liver weights were noted at the time of sacrifice. Representative histological sections were taken from each group in the series.

The following effects of the drugs on normal and carbon tetrachloride-treated animals were observed:

1. Normal Animals

The respiration of the normal animals was shown to be a variable directly dependent upon the age of the animal.

Treatment with the various drugs and with carbon tetrachloride interfered with the normal growth of the animals.

2. Carbon Tetrachloride

The liver respiration was normal at the 95 per cent level of significance. However, in HM flasks, the respiration was directly dependent upon the length of time of administration of carbon tetrachloride. Histologically the livers showed evidence of a beginning cirrhotic lesion accompanied by fatty change and energetic mitotic activity. Thus the liver appeared able to maintain

normal respiration by means of an active regenerative process which was further evidenced by a significantly enlarged liver.

3. Carbon Tetrachloride Discontinued

Following cessation of carbon tetrachloride injections the general condition of the animals improved as did the histological pattern. The respiration returned to within the normal range in HM flasks but was depressed to a significantly low level in KMIII.

4. Corticotropin

Corticotropin had a depressant action upon liver respiration which was non-significant in both KMIII and HM flasks.

5. Corticotropin and Carbon Tetrachloride

Corticotropin and carbon tetrachloride administered concurrently had a depressant action on liver respiration which was significant in HM flasks but not in KMIII. The livers were

significantly enlarged with cellular changes which advanced to the stage of a beginning cirrhotic lesion.

6. Corticotropin after Carbon Tetrachloride

Corticotropin after carbon tetrachloride resulted in an improved histological pattern and in an improved general condition of the animals. The $\dot{Q}O_2$ values indicated that respiration improved at a rate which was significantly faster than in injured livers receiving no therapy. This effect was evident in KMIII but not in HM flasks.

7. Cortisone

Cortisone caused depression of liver respiration both in KMIII and in HM flasks. Although the histological pattern was normal, the livers were significantly enlarged and the mortality rate was high.

8. Cortisone and Carbon Tetrachloride

Cortisone and carbon tetrachloride administered concurrently caused depression of liver respiration which was significant in KMIII but not in HM flasks. The animals were sickly and histological examination showed a liver lesion more extensive than the one produced with carbon tetrachloride alone. The mortality rate was high.

9. Cortisone after Carbon Tetrachloride

Cortisone after carbon tetrachloride allowed the respiration of the liver to return to within the normal range. The general appearance of the animals improved as did the histological appearance of the livers. The livers remained enlarged, light in color, and showed extensive regenerative activity. The mortality rate was high.

10. Alcohol

Alcohol had no apparent effect upon the respiration or the histological picture of the normal liver. Only the low average weight indicated that the animals were undergoing alcohol therapy.

11. Alcohol and Carbon Tetrachloride

Alcohol and carbon tetrachloride exerted a marked depressive action upon the respiration and produced distinctive alterations in the histological pattern. The cirrhotic lesion was the most marked of the series and the animals had the highest mortality rate.

12. Alcohol after Carbon Tetrachloride

Alcohol after carbon tetrachloride allowed the respiration to return to normal. The livers remained significantly large, but appeared normal except for the presence of an altered cell population and regenerative activity.

13. Methionine

Methionine had no significant effect upon liver respiration of normal animals.

14. Methionine and Carbon Tetrachloride

Methionine and carbon tetrachloride caused depression of liver respiration which was significant in HM flasks but not in KMIII. Extensive

histological alterations were evident, of which fatty infiltration was the most prominent. The degree of toxicity to the liver was comparable to that produced by alcohol and carbon tetrachloride given concurrently.

15. Methionine after Carbon Tetrachloride

Methionine after carbon tetrachloride had no effect in improving either the histological pattern or the depressed level of respiration. However, the liver weight and the animal weight returned to normal.

Thus in conclusion it is seen that carbon tetrachloride, corticotropin, alcohol and methionine had no significant effect upon liver respiration of normal animals. Cortisone caused a significant depression of respiration of normal animals.

Carbon tetrachloride produced an alteration in the histological pattern which advanced in the direction of a cirrhotic lesion. The pronounced regenerative activity which enabled the liver to function normally was reflected in the respiration studies.

Corticotropin, cortisone, alcohol and methionine, each administered concurrently with carbon tetrachloride caused a depression in liver respiration. In addition the histological distortion was more severe than when carbon tetrachloride was administered alone. Corticotropin and cortisone produced comparable depression when administered with carbon tetrachloride, as did alcohol and methionine administered with carbon tetrachloride. However, the levels of depression produced by alcohol and methionine were much lower than those produced by cortisone and corticotropin, although other factors entered into the cortisone and corticotropin effects since these animals showed higher mortality rates.

When carbon tetrachloride was administered for 4 weeks and then discontinued, the liver respiration appeared normal in HM flasks, but was significantly below normal in KMIII. Administration of the drugs following cessation of carbon tetrachloride injections produced similarly equivocal results. Corticotropin, cortisone, methionine and alcohol appeared to aid in the recovery of the liver in KMIII, but only alcohol showed an improvement over no therapy in HM flasks. Corticotropin, cortisone, and methionine appeared in HM flasks to be of less benefit than allowing the

liver to recover without supportive therapy.

The significance of the findings and the utility of the Huston-Martin technique are discussed.

1. Osol, A. and Farrar, G.E., The Dispensatory of the United States, 25th Edition, J.B. Lippencott Company, (1955)
2. Goodman, L.S. and Gilman, A., The Pharmacological Basis of Therapeutics, 2nd Edition, MacMillan (1955)
3. Grollman, A., Pharmacology and Therapeutics, 2nd Edition, Lea and Febiger, (1954)
4. Griffith, W.E., Amer. J. Clin. Nutr., 6, 263, (1958)
5. Popper, H. and Zak, F.G., Amer. J. Med., 24, 593, (1958)
6. Harrow, B., Textbook of Biochemistry, 5th Edition, W.B. Saunders Company, (1951)
7. Leduc, E.H. and Wilson, J.W., A.M.A. Arch. Path., 65, 147, (1958)
8. Maximow, A.A. and Bloom, W., Textbook of Histology, 6th Edition, W.B. Saunders Company, (1952).
9. Stowell, R.E. and Lee, C.S., A.M.A. Arch. Path., 50, 519, (1950)
10. Williams, C.F., Anat. Rec., 3, 629, (1951)
11. Wahi, P.N., Tandon, H.D. and Bharadwaj, T.P., Acta path. microbiol. scand., 37, 305, (1955)
12. Myren, R., Acta path. microbiol. scand., Supp. 116, 5, (1956)
13. Hoffman, J., Himes, M.B., Lapan, S., Riszke, R., and Post, J., A.M.A. Arch. Path., 59, 429, (1955).
14. Balasubrahmanyam, M., J. Path. Bact., 65, 123, (1953).
15. Dianzani, M.U., Biochem. J., 65, 116, (1957).

16. Cameron, G.R. and Karunaratne, W.A.E.,
J. Path. Bact., 42, 1, (1936).
17. Post, J., Himes, M.B., Klein, A. and
Hoffman, J., A.M.A. Arch. Path., 64, 284,
(1957).
18. Islami, A.H., Pack, G.T. and Hubbard, J.C.,
Cancer, 11, 663, (1958).
19. Mann, F.C., Fishback, F.C., Gay, J.G. and
Green, G.F., Arch. Path., 12, 787, (1931).
20. Hoffman, J., Himes, M.B., Klein, A., Poulos,
V., and Post, J., A.M.A. Arch. Path., 62,
(1956)
21. Farber, E., Weser, D.K., Szanto, P.B. and
Popper, H., A.M.A. Arch. Path., 51, 399,
(1951).
22. Christie, G.S. and Judah, J.D., Proc. Roy.
Soc. London, B, 142, 241, (1954).
23. Glynn, L.E. and Himsworth, H.P., Clin. Sci.,
6, 235, (1948)
24. Daniel, P.M. Prichard, M.M.L., and Reynell,
P.C., J. Path. Bact., 64, 61, (1952).
25. Popper, H., Weser, D.K. and Szanto, P.B.,
Proc. Soc. Exper. Biol. & Med., 71, 688, (1949)
26. Calvert, D.N., and Brody, T.M., Fed. Proc.,
#1403, 356, March, (1958).
27. Forbes, J.C., J. Pharm., 65, 287, (1939).
28. Brunschwig, A., Johnson, C. and Nichols, S.,
Proc. Soc. Exper. Biol. & Med., 60, 388,
(1945).
29. Vorhaus, E.F. and Vorhaus, L.J.,
Gastroenterology, 26, 887, (1954).
30. Popper, H., Weser, D.K. and Szanto, P.B.,
Proc. Soc. Exper. Biol. & Med., 71, 688,
(1949).
31. Rachmilewitz, M., Stein, Y., Arnovitch, J.,
and Grossowicz, N., A.M.A. Arch. intern.
Med., 101, 1118, (1958).
32. Yamamoto, R.S., Okuda, K., and Chow, B.F.,
Proc. Soc. Exper. Biol. & Med., 94, No. 3,
(1957).

33. Gabuzda, G.J., Amer. J. Clin. Nutr., 6, 263, (1958).
34. Ruebner, B., Bramhall, J.L. and Berry, G.R., A.M.A. Arch. Path., 66, 165, (1958).
35. Sidransky, H. and Farber, E., A.M.A. Arch. Path., 66, 119, (1958).
36. Zilversmit, D.B. and DiLuzio, N.R., Amer. J. Clin. Nutr., 6, 235, (1958).
37. Artom, C., Amer. J. Clin. Nutr., 6, 221, (1958).
38. Rees, E.D. and Kline, D.L., Amer. J. Physiol., 193, 431, (1958).
39. Olson, R.E., Jablonski, J.R. and Taylor, E., Amer. J. Clin. Nutr., 6, 111, (1958).
40. Sborov, V.M., Amer. J. dig. Dis., 3, 94, (1958).
41. Harper, A.E., Amer. J. Clin. Nutr., 6, 242 (1958).
42. Channon, H.J. and Wilkinson, H., Biochem. J., 29, 350, (1935).
43. Beeston, A.W. and Channon, H.J., Biochem. J., 30, 280, (1936).
44. Tucker, H.F. and Eckstein, H.C., J. biol. Chem., 121, 479, (1937).
45. Tucker, H.F. and Eckstein, H.C., J. biol. Chem., 126, 117, (1938).
46. Channon, H.J., Loach, J.V., Loizedes, P.A., Manifold, M.C. and Soliman, G., Biochem. J., 32, 976, (1938).
47. Tucker, H.F., Treadwell, C.R. and Eckstein, H.C., J. biol. Chem., 135, 85, (1940).
48. Treadwell, C.R., Groothuis, H.G. and Eckstein, H.C., J. biol. Chem., 142, 653, (1942).
49. Horning, M.G. and Eckstein, H.C., J. biol. Chem., 155, 49, (1944).
50. Griffith, W.H. and Mulford, D.J., J. Nutr., 21, 633, (1941).

51. Mulford, D.J. and Griffith, W.H.,
J. Nutr., 23, 91, (1942).
52. Harper, A.E., Benton, D.A., Winje, H.W.,
and Elvehjem, C.A., J. biol. Chem., 214,
677, (1955).
53. Cohen, S.I., Schmatolla, E., Bevans, M.
and Patek, J. Jr., Arch. intern. Med.,
101, 291, (1958).
54. Roth, J.S. and Milstein, S.W., Arch. Biochem.
& Biophysics, 70, 392, (1957).
55. Munro, H.N. and Mukerji, D., Biochem. J.,
69, 321, (1958).
56. Tucker, H.F. and Eckstein, H.C., J. biol.
Chem., 121, 479, (1937).
57. Channon, H.J., Manifold, H.C. and Platt, A.P.,
Biochem. J., 32, 969, (1938).
58. Best, C.H. and Ridout, J.H., J. Physiol.
97, 489, (1940).
59. Singal, S.A. and Eckstein, H.C., Proc. Soc.
Exper. Biol. & Med., 41, 512, (1937).
60. Guild, W.R., Young, J.V. and Merrill, J.P.,
Ann. intern. Med., 48, 1221, (1958).
61. Zieve, L., Ann. intern. Med., 48, 471,
(1958).
62. Griffith, W.E., Amer. J. Clin. Nutr.,
6, 263, (1958).
63. Aterman, K., A.M.A. Arch. Path., 57, 12,
(1954).
64. Aterman, K., A.M.A. Arch. Path., 57, 26,
(1954).
65. Cavellero, C., Sala, G., Amira, A. and
Borasi, M., Lancet, 260, 55, (1951).
66. Diengott, D., and Ungar, H., A.M.A. Arch.
Path., 58, 449, (1954).
67. Aterman, K. and Ahmad, M.D., Lancet, 1,
71, (1953).

68. Brown, H., Jager, B.V. and Tyler, P.H., Amer. J. Med., 10, 170, (1951).
69. Zoeckler, S.J., Gastroenterology, 26, 678, (1954).
70. Williams, C.F. and Flink, E.B., J. Lab. Clin. Med., 39, 388, (1952).
71. Wahi, P.N., Tandon, H.D. and Bharadwaj, T.P., A.M.A. Arch. Path., 62, 200, (1956).
72. Wahi, P.N., Tandon, H.D. and Bharadwaj, T.P., A.M.A. Arch. Path., 62, 215, (1956).
73. Bach, S.J., Carter, S.B. and Killop, J.S., Biochim. biophys. acta, 28, 168, (1958).
74. Dunn, C.E., Bass, A.D. and Mc Ardle, A.H., Exp. Cell Res., 14, 23, (1958).
75. Ashmore, J., Gahill, G.F., Gillman, R., and Renold, A.E., Endocrinology, 63, No. 5, (1958).
76. Pessar, T. and Helsing, J.W., Ann. intern. Med., 48, 1254, (1958).
77. Wool, I.G. and Goldstein, M.C., Amer. J. Physiol., 193, 65, (1958).
78. Lieberman, S. and Teich, S., Pharmacol. Rev., 5, 312, (1953).
79. Roberts, S. and Szego, C.M., Physiol. Rev., 33, 593, (1953).
80. Umbreit, W.W., Ann. N.Y. Acad. Sc., 56, 569, (1951).
81. Gold, N.I. and Sturgis, S.H., Endocrinology, 56, 636, (1955).
82. Schwartz, T.B., Robertson, M.C. and Holmes, L.B., Endocrinology, 58, 453, (1956).
83. Lacroix, E. and Leusen, I., Arch. int. Pharmacodyn., 114, 103, (1958).
84. Huston, M.J. and Martin, A.W., Proc. Soc. exp. Biol. N.Y., 86, 103, (1954).

85. Huston, M.J. and Martin, A.W.,
Arch. int. Pharmacodyn., 101, 349, (1955).
 86. Calvert, D.W. and Huston, M.J.,
Arch. int. Pharmacodyn., 112, 45, (1957).
 87. Moore, K.E., Murray, J.R., and Huston, M.J.,
To be published.
 88. Umbreit, W.W., Burris, R.H. and Stauffer, J.F.,
Manometric Techniques and Tissue Metabolism,
2nd Edition, Burgess.
 89. Krebs, H.A., Biochim. biophys. acta, 4, 249,
(1950).
 90. Martin, A.W., Endocrinology, 30, 624,
(1942).
 91. Kenny, J.F. and Keeping, E.S., Mathematics
of Statistics, Part I, 3rd Edition, D. Van
Nostrand, (1954).
 92. Rodnight, R. and McIlwain, H., Biochem. J.
57, 649, (1954).
 93. Drabkin, E.L. and Marsh, J.B., J. biol.
Chem., 221, 71, (1956).
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APPEND IX

TABLE i

RESPIRATION OF NORMAL RAT LIVER SLICES IN KMIII

QO₂ values are given in ml O₂ per gram wet weight per hour at time of setting.

The days represent the time elapsed from the start of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
1	2.92	52	3.12	75	3.06
1	2.80	52	2.82	75	2.86
1	3.09	54	3.38	75	3.25
1	3.22	54	2.96	75	3.63
1	3.04	54	2.92	75	3.42
1	2.69	58	3.24	80	4.73
13	3.10	58	2.58	80	4.64
13	2.28	58	2.61	80	3.35
13	1.59	61	2.95	82	3.21
28	3.02	61	2.44	82	3.29
28	2.59	61	3.50	82	3.00
28	2.61	64	4.08	82	3.38
35	2.03	64	2.92	82	3.32
35.	2.15	64	3.01	82	3.04
44	4.12	67	2.75	83	3.21
44	3.22	67	2.65	83	3.19
44	3.03	67	2.72	83	3.45
45	2.48	69	3.04	83	3.55
45	2.95	69	3.05	83	3.34
45	3.01	69	2.38	83	3.38
52	3.12	75	3.50		

Mean QO₂ value 3.08

s.d.m. 0.52

TABLE ii

RESPIRATION OF NORMAL RAT LIVER SLICES IN HM FLASKS

QO₂ values are given in ml O₂ per gram wet weight per hour at time of setting.

The number of days represents the time elapsed from the start of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
1	2.65	45	2.49	69	3.60
1	3.42	52	3.06	75	3.14
1	3.02	52	3.47	75	2.58
1	3.10	52	3.37	75	2.62
1	3.10	54	2.80	75	3.70
1	2.60	54	2.72	75	3.09
13	2.02	54	2.72	75	3.36
28	3.28	58	4.00	80	3.37
28	3.20	58	3.23	80	3.38
28	3.34	58	2.86	82	3.38
35	2.20	61	3.50	82	3.20
35	2.41	61	3.44	82	3.08
35	2.98	61	3.55	82	3.18
44	3.01	64	3.75	82	3.21
44	2.78	64	3.20	82	3.09
44	2.83	67	3.22	83	3.29
45	3.09	67	2.82	83	3.10
45	3.25	69	3.70	83	3.42
				83	3.78
				83	3.20
				83	3.84

Mean QO₂ value 3.14

s.d.m. 0.40

TABLE iii

THE EFFECT OF CARBON TETRACHLORIDE ON RESPIRATION OF
RAT LIVER SLICES IN KMIII.

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The number of days represents the time elapsed from
the start of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
1	2.28	30	2.40	48	3.58
8	2.96	30	2.44	48	3.70
8	2.76	31	2.72	48	3.42
8	2.32	31	2.70	49	3.03
21	2.71	31	2.72	49	2.16
21	3.02	32	3.70	49	2.50
21	2.91	32	3.30	52	2.98
23	2.82	32	2.78	52	2.88
23	2.90	33	3.38	55	3.78
23	2.68	33	3.55	55	3.63
25	3.13	33	3.10	55	3.73
25	2.35	38	2.85	61	3.52
25	2.56	38	3.20	61	3.57
30	1.84	38	2.60	61	2.94

Mean QO₂ value 2.96

s.d.m. 0.48

TABLE iv

THE EFFECT OF CARBON TETRACHLORIDE ON RESPIRATION OF RAT LIVER SLICES IN HM FLASKS.

QO₂ values are given in ml O₂ per gram wet weight per hour at time of setting.

The number of days represents the time elapsed from the start of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
1	2.62	30	3.15	48	4.49
1	1.45	30	3.20	48	3.96
8	3.12	30	2.93	48	4.35
8	3.18	31	2.92	49	2.08
8	3.41	31	2.49	49	2.99
21	1.40	31	2.50	49	1.77
21	2.08	32	3.12	52	3.38
21	2.10	32	2.73	52	3.03
23	3.92	32	3.08	52	3.80
23	4.43	33	3.58	55	3.47
23	3.13	33	2.98	55	2.53
25	3.06	33	3.88	55	3.48
25	2.66	38	3.50	61	3.22
25	2.78	38	3.65	61	3.22
		38	3.67	61	3.58
Mean QO ₂ value		3.09			
s.d.m.		0.70			

TABLE v

THE EFFECT OF CARBON TETRACHLORIDE DISCONTINUED ON
RESPIRATION OF RAT LIVER SLICES IN KMIII.

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The number of days represents the time elapsed
from the start of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
40	2.48	62	2.09	87	2.42
40	2.60	62	2.46	93	1.40
40	2.51	70	2.93	93	2.00
48	3.50	70	2.64	93	2.49
48	3.00	70	2.90	98	2.58
48	2.98	79	3.50	98	2.28
55	2.88	79	2.90	98	2.20
55	2.79	79	3.22	103	2.83
55	3.10	87	3.24	103	2.60
62	2.62	87	2.58	103	1.92

Mean QO₂ value 2.66

s.d.m. 0.45

TABLE vi

THE EFFECT OF CARBON TETRACHLORIDE DISCONTINUED
ON RESPIRATION OF RAT LIVER SLICES IN HM FLASKS

QO_2 values are given in ml O_2 per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the
start of injections.

Days	QO_2	Days	QO_2	Days	QO_2
40	1.78	62	1.56	87	2.82
40	2.37	62	3.12	93	2.10
40	2.75	70	3.70	93	2.39
48	2.85	70	3.98	98	3.63
48	2.80	70	3.53	98	3.38
48	3.07	79	3.58	98	2.70
55	2.72	79	3.57	103	3.41
55	2.74	79	3.53	103	1.91
55	2.19	87	4.03	103	3.28
62	3.23	87	2.82		

Mean QO_2 value 2.95

s.d.m. 0.64

TABLE vii

THE EFFECT OF CORTICOTROPIN ON THE RESPIRATION
OF NORMAL RAT LIVER SLICES IN KMIII

QO₂ values are given in ml O₂ per gram wet weight per hour at time of setting.

The number of days represents the time elapsed from the start of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
9	1.82	39	2.30	57	2.27
9	2.28	39	2.63	64	2.17
9	2.19	42	2.98	64	2.30
24	2.98	42	2.83	75	3.00
24	3.10	42	2.40	75	2.85
24	2.76	48	2.68	75	2.93
31	2.35	48	2.77	84	2.95
31	2.63	48	2.60	84	2.67
31	3.13	51	3.02	84	2.78
34	3.03	51	2.53	89	2.45
34	2.76	51	2.66	89	2.93
34	2.08	57	3.03	89	2.69
39	2.78	57	2.99	95	3.17
				95	2.04

Mean QO₂ value 2.66

s.d.m. 0.33

TABLE viii

THE EFFECT OF CORTICOTROPIN ON THE RESPIRATION OF
NORMAL RAT LIVER SLICES IN HM FLASKS

QO_2 values are given in ml O_2 per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the
start of injections.

Days	QO_2	Days	QO_2	Days	QO_2
9	2.50	39	1.48	64	3.13
9	2.42	42	3.41	64	3.14
9	2.17	42	2.96	75	3.43
24	3.26	42	2.98	75	3.38
24	3.09	48	3.09	75	3.17
24	2.82	48	2.70	84	4.12
31	3.38	48	2.70	84	3.34
31	3.20	51	3.50	83	3.41
31	2.70	51	3.72	89	2.50
34	3.26	51	3.52	89	3.36
34	2.12	57	2.54	89	3.30
39	2.10	57	2.66	95	3.12
39	2.40	64	3.23	95	2.93
				95	2.32

Mean QO_2 value 2.96

s.d.m. 0.52

TABLE ix

THE EFFECT OF CORTICOTROPIN AND CARBON TETRACHLORIDE ON
RESPIRATION OF RAT LIVER SLICES IN KMIII

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
36	1.56	59	2.55	91	1.88
36	2.78	67	2.38	91	3.63
45	2.76	67	2.41	91	1.98
45	2.50	67	1.81	97	3.21
45	2.52	77	2.40	97	3.14
50	3.29	77	2.63	97	2.96
50	2.82	77	2.30	100	2.65
50	2.69	86	1.25	100	1.85
59	2.83	86	1.90	100	2.42
59	2.99	86	1.28		
Mean QO ₂ value		2.46			
s.d.m.		0.58			

TABLE x

THE EFFECT OF CORTICOTROPIN AND CARBON TETRACHLORIDE
ON RESPIRATION OF RAT LIVER SLICES IN HM FLASKS

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
36	2.29	59	3.40	86	1.52
36	2.65	59	3.08	91	3.30
45	3.35	67	3.37	91	3.43
45	3.51	67	2.95	91	2.70
45	3.62	67	3.08	97	3.09
50	3.63	77	2.85	97	3.15
50	3.70	77	2.42	97	2.78
50	3.47	77	2.20	100	2.20
59	3.00	86	1.56	100	2.67
Mean QO ₂ value		2.92			
s.d.m.		0.58			

TABLE xi

THE EFFECT OF CORTICOTROPIN AFTER CARBON TETRACHLORIDE
DISCONTINUED ON THE RESPIRATION OF RAT LIVER SLICES
IN KMIII.

QO₂ values are given in ml O₂ per gram wet weight per
hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
41	2.88	63	2.38	93	2.83
41	1.49	63	1.89	93	3.00
41	3.03	70	2.69	98	2.22
48	2.46	70	2.65	98	2.13
48	2.68	79	2.78	98	1.83
48	2.52	79	2.75	103	3.15
55	3.11	79	2.80	103	2.20
55	2.48	84	2.70	103	2.03
55	2.92	84	2.52	103	2.81
63	2.75	84	2.33		

Mean QO₂ value 2.55

s.d.m. 0.42

TABLE xii

THE EFFECT OF CORTICOTROPIN AFTER CARBON TETRACHLORIDE
DISCONTINUED ON RESPIRATION OF RAT LIVER SLICES IN
HM FLASKS.

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
41	2.03	63	2.45	84	3.36
41	1.60	63	2.76	84	2.63
48	2.82	70	3.10	93	3.18
48	2.49	70	2.18	93	3.00
48	2.42	70	2.83	93	2.95
55	2.63	79	2.63	98	3.11
55	2.72	79	2.42	98	3.08
55	2.83	79	3.59	98	0.90
63	2.71	84	3.10	103	1.80
				103	1.92
Mean QO ₂		2.62			
s.d.m.		0.78			

TABLE xiii

THE EFFECT OF CORTISONE ON THE RESPIRATION OF
NORMAL RAT LIVER SLICES IN KMIII

QO_2 values are given in ml O_2 per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the
start of injections.

Days	QO_2	Days	QO_2	Days	QO_2
21	3.38	42	3.13	58	2.67
21	3.22	42	3.12	63	4.49
21	3.10	51	3.39	63	2.75
29	3.52	51	3.20	63	2.62
29	3.31	51	2.48	70	2.78
29	3.30	54	3.38	70	2.51
38	3.75	54	3.43	70	2.60
38	3.22	54	3.78	75	3.60
38	2.82	58	3.60	75	2.18
42	2.98	58	3.53	75	2.19

Mean QO_2 3.13

s.d.m. 0.50

TABLE xiv

THE EFFECT OF CORTISONE^E ON RESPIRATION OF
NORMAL RAT LIVER SLICES IN HM FLASKS

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the
start of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
21	3.38	42	2.65	58	2.51
21	2.85	42	2.25	63	2.95
21	2.85	51	2.50	63	2.56
29	3.18	51	3.53	63	3.08
29	2.64	51	3.31	70	2.95
29	3.14	54	3.27	70	2.70
38	3.40	54	2.07	70	2.69
38	3.73	54	2.82	75	3.12
38	3.02	58	3.36	75	2.58
42	3.42	58	2.80		
Mean QO ₂		2.94			
s.d.m.		0.39			

TABLE xv

THE EFFECT OF CORTISONE AND CARBON TETRACHLORIDE ON
THE RESPIRATION OF RAT LIVER SLICES IN KMIII

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
22	3.58	39	3.23	54	2.21
22	2.73	39	3.12	59	2.88
22	2.50	41	3.04	60	3.08
29	2.09	41	2.81	60	3.10
29	2.70	41	2.58	66	3.45
29	2.91	51	2.83	66	2.92
37	4.90	51	3.49	66	3.03
37	3.26	51	2.83	68	3.93
37	3.60	54	3.00	68	3.24
39	3.30	54	2.70	68	2.97

Mean QO₂ 3.07

s.d.m. 0.52

TABLE xvi

THE EFFECT OF CORTISONE AND CARBON TETRACHLORIDE
ON RESPIRATION OF RAT LIVER SLICES IN
HM FLASKS

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
22	3.29	39	2.30	54	2.10
22	3.38	39	2.05	60	3.12
22	2.67	41	3.50	60	2.60
29	3.75	41	3.10	60	3.38
29	3.19	41	3.58	66	3.23
29	3.10	51	2.94	66	3.43
37	3.12	51	3.03	66	2.80
37	3.06	51	3.18	68	3.70
37	3.01	54	2.51	68	3.96
39	2.30	54	3.09	68	3.29
Mean QO ₂		3.06			
s.d.m.		0.67			

TABLE xvii

THE EFFECT OF CORTISONE AFTER CARBON TETRACHLORIDE
DISCONTINUED ON RESPIRATION OF RAT LIVER SLICES
IN KMIII

QO_2 values are given in ml O_2 per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the
start of injections.

Days	QO_2	Days	QO_2	Days	QO_2
28	2.62	44	3.65	56	3.06
28	2.60	44	3.30	56	3.02
28	3.20	44	2.82	56	3.03
33	3.36	44	3.23	63	2.20
33	2.78	44	3.32	63	3.12
33	2.58	44	3.42	63	2.75
43	3.80	53	3.30	68	3.43
43	2.77	53	3.25	68	3.42
43	1.82	53	2.70	68	3.42
Mean QO_2		3.04			
s.d.m.		0.44			

TABLE xviii

THE EFFECT OF CORTISONE AFTER CARBON TETRACHLORIDE
DISCONTINUED ON RESPIRATION OF RAT LIVER SLICES IN
HM FLASKS

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
28	2.94	44	3.21	56	2.20
28	2.52	44	2.95	56	2.46
28	2.80	44	3.00	63	3.18
33	3.85	44	2.30	63	3.10
33	3.73	44	3.00	63	3.00
33	2.96	53	2.71	68	3.20
43	2.84	53	2.72	68	3.03
43	2.08	53	2.61	68	3.02
44	3.41	56	2.34		

Mean QO₂ 2.89

s.d.m. 0.42

TABLE xix

THE EFFECT OF ALCOHOL ON THE RESPIRATION OF NORMAL
RAT LIVER SLICES IN KMIII

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
10	2.88	45	2.75	77	2.90
10	1.82	51	2.12	77	3.58
10	2.12	51	2.58	86	4.31
24	3.09	51	2.95	86	3.46
24	2.63	51	3.25	86	3.04
24	1.35	51	2.53	92	3.06
32	2.48	51	2.85	92	2.67
32	2.74	62	3.19	92	2.68
32	2.48	62	3.09	97	3.12
38	3.82	62	2.98	97	2.33
38	3.68	69	2.73	101	1.65
38	3.18	69	3.00	101	2.72
45	2.32	69	2.80	101	2.49
45	2.82	77	3.64		
Mean QO ₂		2.80			
s.d.m.		0.56			

TABLE xx

THE EFFECT OF ALCOHOL ON THE RESPIRATION OF NORMAL
RAT LIVER SLICES IN HM FLASKS

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
10	2.38	45	3.32	69	3.66
10	2.65	45	3.01	77	3.77
24	3.58	45	3.20	77	4.00
24	3.53	51	3.38	86	3.21
24	3.05	51	2.96	86	2.77
32	4.24	51	3.18	86	2.82
32	4.17	51	3.38	92	3.08
32	4.00	51	3.58	92	2.89
38	3.50	51	3.03	97	2.63
38	3.48	62	3.63	97	3.32
38	3.00	62	4.11	101	3.95
42	3.16	62	3.80	101	3.97
42	3.26	69	3.39	101	2.28
42	3.33	69	3.50		

Mean QO₂ 3.34

s.d.m. 0.47

TABLE xxi

THE EFFECT OF ALCOHOL AND CARBON TETRACHLORIDE ON
RESPIRATION OF RAT LIVER SLICES IN KMIII

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the
start of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
37	3.28	50	2.83	68	2.46
37	3.12	50	3.48	68	2.20
37	2.96	50	2.82	68	2.30
45	1.63	61	1.81	78	2.37
45	1.70	61	2.88	78	2.76
45	1.67	61	2.66	78	2.63
Mean QO ₂		2.53			
s.d.m.		0.61			

TABLE xxi

THE EFFECT OF ALCOHOL AND CARBON TETRACHLORIDE ON
THE RESPIRATION OF RAT LIVER SLICES IN HM FLASKS.

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days.	QO ₂
37	3.20	50	2.96	68	2.60
37	3.28	50	2.43	68	2.58
37	3.04	50	3.10	68	1.73
45	1.29	61	2.91	78	2.59
45	1.48	61	2.71	78	2.58
45	1.20	61	3.22	78	2.20

Mean QO₂ value 2.50

s.d.m. 0.65

TABLE xxiii

THE EFFECT OF ALCOHOL AFTER CARBON TETRACHLORIDE
DISCONTINUED ON THE RESPIRATION OF RAT LIVER SLICES
IN KMIII.

QO_2 values are given in ml O_2 per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO_2	Days	QO_2	Days	QO_2
41	2.18	63	1.72	89	2.99
41	3.10	63	2.33	89	2.75
41	2.28	63	2.32	94	3.60
48	2.80	71	3.48	94	2.71
48	2.35	71	3.58	94	2.90
48	2.76	71	3.20	99	3.32
56	3.08	83	1.53	99	2.81
56	2.70	83	1.46	99	3.25
56	2.65	89	3.72		
Mean QO_2		2.75			
s.d.m.		0.60			

TABLE xxiv

THE EFFECT OF ALCOHOL AFTER CARBON TETRACHLORIDE
DISCONTINUED ON THE RESPIRATION OF RAT LIVER SLICES
IN HM FLASKS.

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
41	2.62	63	4.08	89	3.50
41	2.41	63	3.38	94	3.63
41	2.21	63	3.32	94	3.48
48	3.40	71	3.70	94	3.52
48	3.10	71	3.68	99	4.40
56	3.60	71	2.97	99	3.55
56	3.11	89	2.40	99	1.60
56	3.41	89	3.32		
Mean QO ₂		3.23			
s.d.m.		0.62			

TABLE xxv

THE EFFECT OF METHIONINE ON RESPIRATION OF
NORMAL RAT LIVER SLICES IN KMIII

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
10	2.46	42	1.85	77	3.62
10	2.62	47	3.08	77	3.09
10	2.42	47	3.00	77	2.70
25	3.38	47	2.73	86	3.31
25	3.23	51	2.69	86	2.80
25	2.78	51	2.42	86	2.60
32	2.42	51	2.41	91	2.27
32	2.51	51	2.02	91	2.33
38	1.70	61	1.88	97	3.58
38	3.01	61	2.77	97	3.10
38	2.70	61	2.80	97	3.20
42	2.78	68	3.10	100	2.75
42	2.95	68	2.35	100	2.77
				100	2.08

Mean QO₂ value 2.71

s.d.m. 0.33

TABLE xxvi

THE EFFECT OF METHIONINE ON THE RESPIRATION OF
NORMAL RAT LIVER SLICES IN HM FLASKS

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
10	3.23	47	3.39	77	2.71
10	3.05	47	3.23	77	2.30
10	2.64	47	3.02	77	2.58
25	3.75	51	2.83	86	3.08
25	3.12	51	3.22	86	3.32
25	3.47	51	3.52	86	3.41
32	3.27	51	2.67	91	3.16
32	3.08	51	2.60	91	2.80
32	3.80	51	2.30	91	1.59
38	3.36	61	3.18	97	3.41
38	2.00	61	2.93	97	3.51
42	3.25	68	3.73	100	3.70
42	3.20	68	2.61	100	3.78
42	3.33	68	2.59	100	3.25
Mean QO ₂		3.07			
s.d.m.		0.60			

TABLE xxvii

THE EFFECT OF METHIONINE AND CARBON TETRACHLORIDE ON
THE RESPIRATION OF RAT LIVER SLICES IN KMIII.

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
40	3.02	69	1.78	86	2.72
40	2.61	69	2.68	92	2.72
40	2.48	69	1.08	92	2.86
47	1.52	78	2.28	92	2.18
47	2.12	78	2.18	97	2.43
62	2.21	78	2.04	97	2.91
62	1.90	86	2.85	97	1.70
62	2.17	86	2.28	100	1.29

Mean QO₂ 2.25

s.d.m. 0.51

TABLE xxviii

THE EFFECT OF METHIONINE AND CARBON TETRACHLORIDE
ON THE RESPIRATION OF RAT LIVER SLICES IN
HM FLASKS

QO_2 values are given in ml O_2 per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO_2	Days	QO_2	Days	QO_2
40	2.80	62	2.82	86	2.54
40	2.13	69	2.85	86	3.20
40	2.95	69	3.00	92	4.00
47	1.15	69	1.63	92	3.10
47	2.65	78	2.46	97	3.70
47	2.23	78	2.70	97	3.80
62	2.53	78	2.62	97	3.30
62	3.03	86	3.02	100	2.55
				100	3.76

Mean QO_2 2.79

s.d.m. 0.75

TABLE xxix

THE EFFECT OF METHIONINE AFTER CARBON TETRACHLORIDE
DISCONTINUED ON THE RESPIRATION OF RAT LIVER
SLICES IN KMIII

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
41	2.52	63	2.70	83	2.52
41	2.86	63	2.61	90	2.78
41	2.56	63	2.83	90	2.09
49	2.18	71	2.12	99	3.15
49	1.78	71	3.03	104	3.23
49	1.42	71	2.70	104	2.77
56	2.48	83	1.83	104	1.68
56	3.77	83	2.20	104	2.02
				104	2.70

Mean QO₂ value 2.50

s.d.m. 0.53

THE CHAIRMAN OF THE BOARD OF DIRECTORS
OF THE COMPANY
1940

THE CHAIRMAN OF THE BOARD OF DIRECTORS
OF THE COMPANY
1940

THE CHAIRMAN OF THE BOARD OF DIRECTORS
OF THE COMPANY
1940

1940	1939	1938	1937	1936	1935
100.0	100.0	100.0	100.0	100.0	100.0
100.0	100.0	100.0	100.0	100.0	100.0
100.0	100.0	100.0	100.0	100.0	100.0
100.0	100.0	100.0	100.0	100.0	100.0
100.0	100.0	100.0	100.0	100.0	100.0
100.0	100.0	100.0	100.0	100.0	100.0
100.0	100.0	100.0	100.0	100.0	100.0
100.0	100.0	100.0	100.0	100.0	100.0
100.0	100.0	100.0	100.0	100.0	100.0
100.0	100.0	100.0	100.0	100.0	100.0

100.0
100.0

TABLE xxx

THE EFFECT OF METHIONINE AFTER CARBON TETRACHLORIDE
DISCONTINUED ON THE RESPIRATION OF RAT LIVER
SLICES IN HM FLASKS

QO₂ values are given in ml O₂ per gram wet weight
per hour at time of setting.

The days represent the time elapsed from the start
of injections.

Days	QO ₂	Days	QO ₂	Days	QO ₂
41	2.92	63	3.18	90	3.19
41	2.92	63	2.70	99	2.42
41	3.80	71	3.03	99	2.68
49	1.97	71	3.18	99	2.43
49	2.42	71	3.05	104	3.83
49	2.32	83	1.11	104	3.30
56	2.20	83	1.70	104	1.17
56	2.38	83	1.82	104	2.36
56	2.12	90	3.13	104	1.75
63	2.83	90	2.98	104	2.90

Mean QO₂ 2.59

s.d.m. 0.66

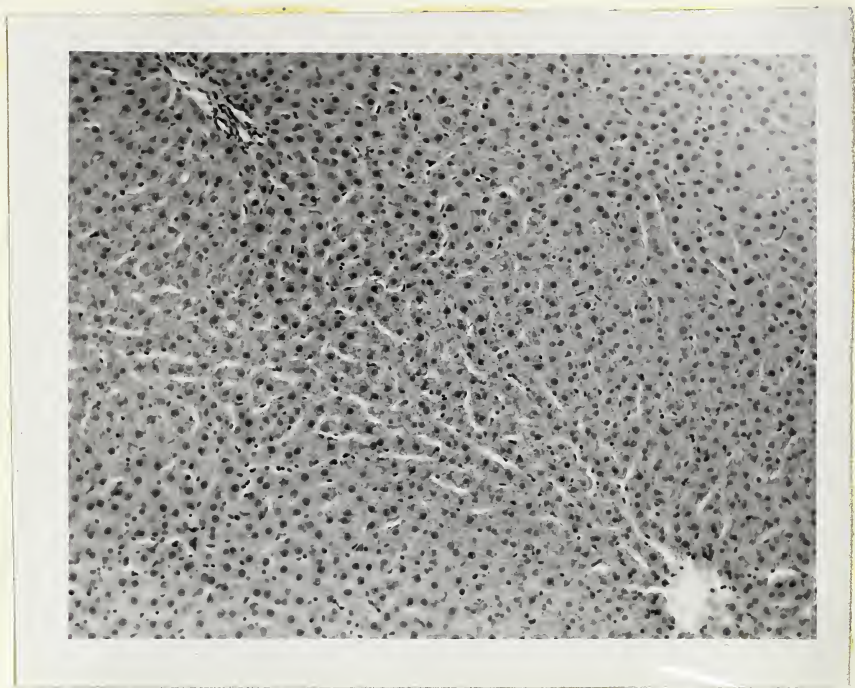


FIGURE i

HISTOLOGICAL STRUCTURE OF NORMAL
RAT LIVER

(magnification 80X)

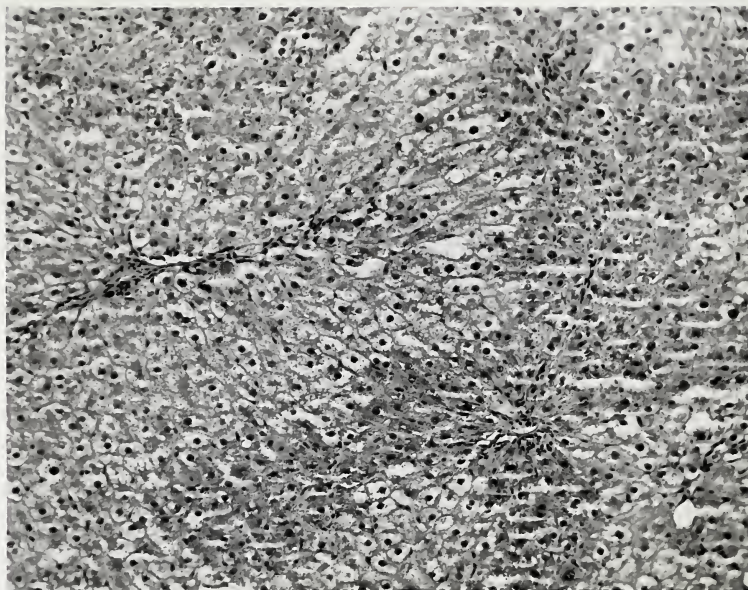


FIGURE ii

HISTOLOGICAL STRUCTURE OF LIVER OF
CARBON TETRACHLORIDE-TREATED RAT

The following histological alterations are seen: Lobular disarray, centrilobular necrosis, focal necrosis, hydropic change, portal inflammatory infiltrate, binuclear cells, cirrhosis, fatty change and regenerative activity.

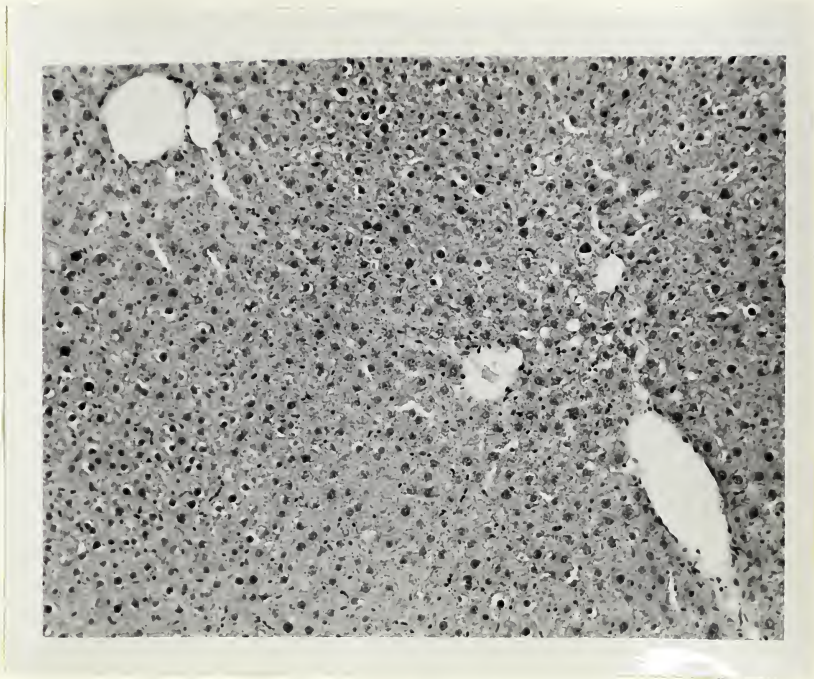


FIGURE iii

THE HISTOLOGICAL STRUCTURE OF RAT LIVER
AFTER CARBON TETRACHLORIDE DISCONTINUED

The following histological alterations are noted: portal inflammatory infiltrate, lobular disarray, and regenerative activity.

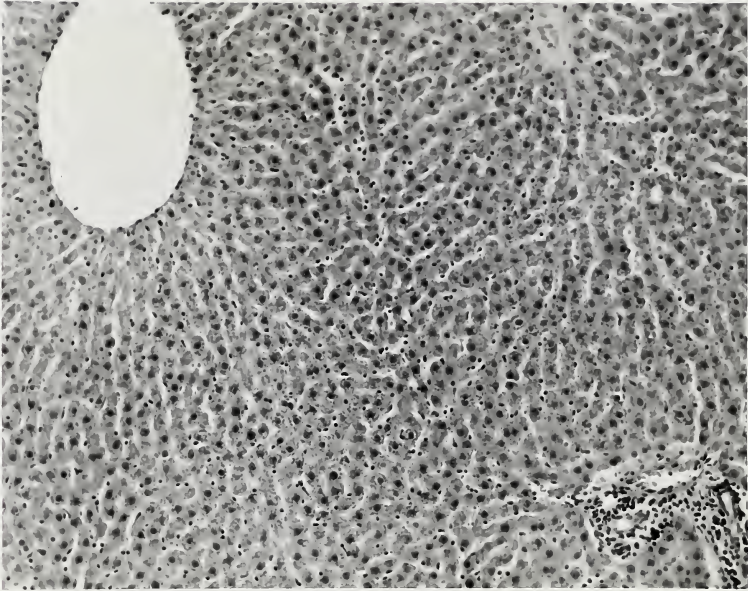


FIGURE iv

THE HISTOLOGICAL STRUCTURE OF NORMAL RAT
LIVER RECEIVING CORTICOTROPIN

The liver is essentially normal, but the following alterations are noted: binuclear cells, lobular disarray, regenerative activity, and portal inflammatory infiltrate.

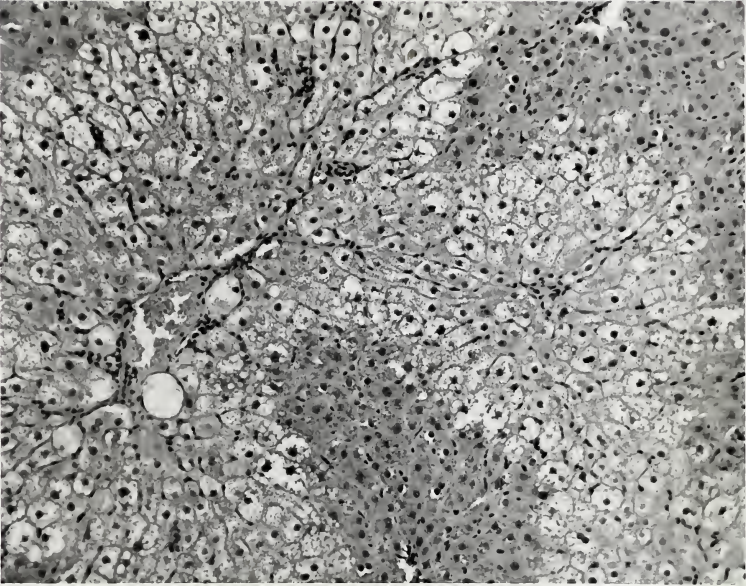


FIGURE v

THE HISTOLOGICAL STRUCTURE OF THE LIVER
OF RAT RECEIVING CORTICOTROPIN AND
CARBON TETRACHLORIDE

The following histological alterations are noted: centrolobular necrosis, lobular disarray, hydropic change (central), fat infiltration, binuclear cells, portal inflammatory infiltrate, regenerative activity, and beginning cirrhosis.

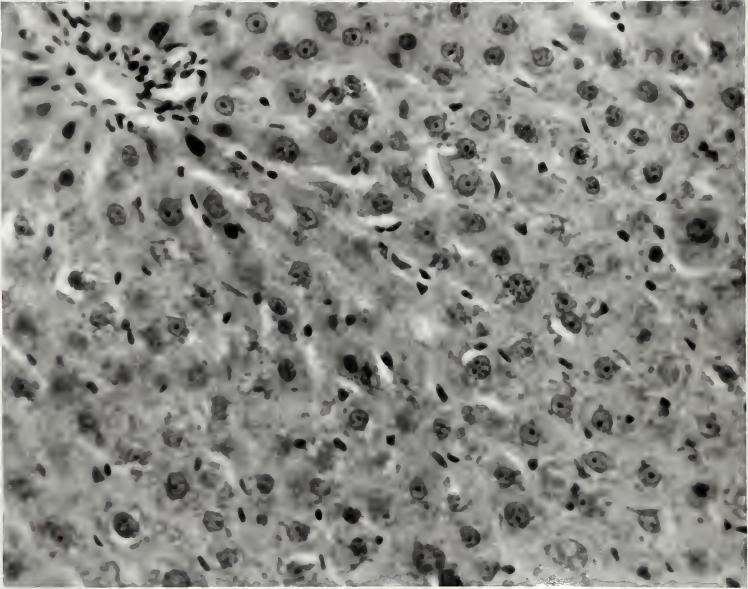


FIGURE vi

THE HISTOLOGICAL STRUCTURE OF THE LIVER OF
A RAT RECEIVING CORTICOTROPIN AFTER
CARBON TETRACHLORIDE DISCONTINUED

The following histological alterations are present: binuclear cells, lobular disarray, regenerative activity, and portal inflammatory infiltrate.

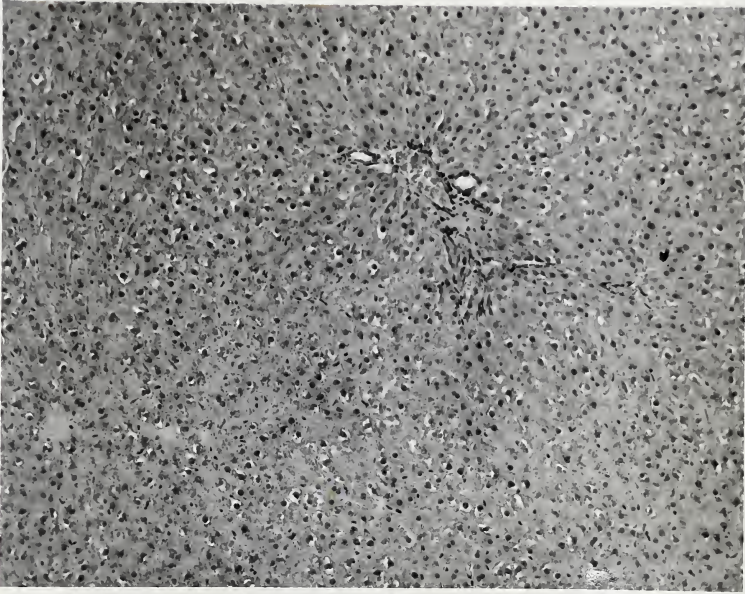


FIGURE vii

THE HISTOLOGICAL STRUCTURE OF THE LIVER
OF A NORMAL RAT RECEIVING CORTISONE

Binuclear cells are evident, but the liver is
essentially normal.

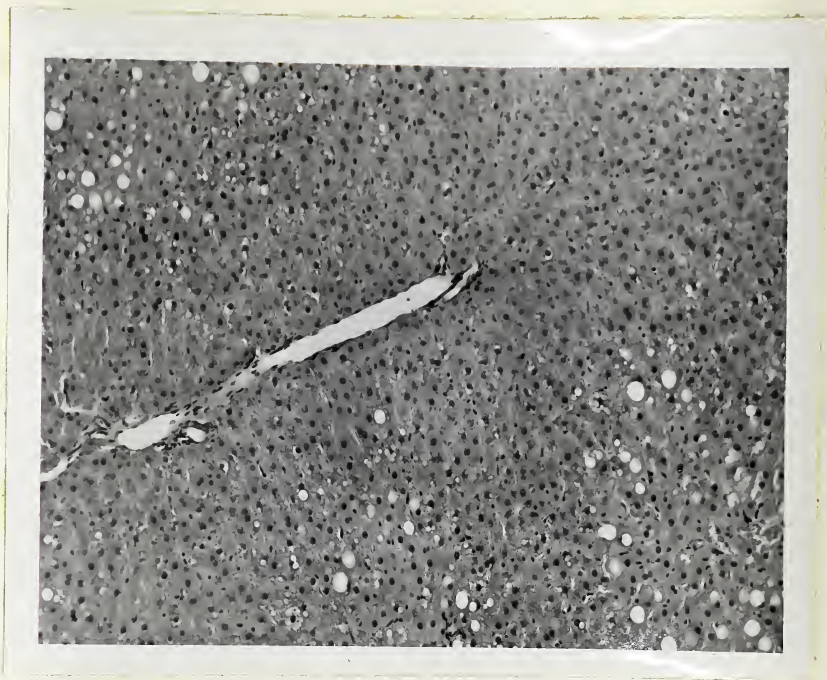


FIGURE viii

THE HISTOLOGICAL STRUCTURE OF THE LIVER
OF A RAT RECEIVING CORTISONE AND
CARBON TETRACHLORIDE

The following alterations are present:
centrolobular necrosis, focal necrosis, lobular
disarray, hydropic change (central), portal
inflammatory infiltrate, dispersed cytoplasmic
basophilia, regenerative activity and fatty
infiltration.

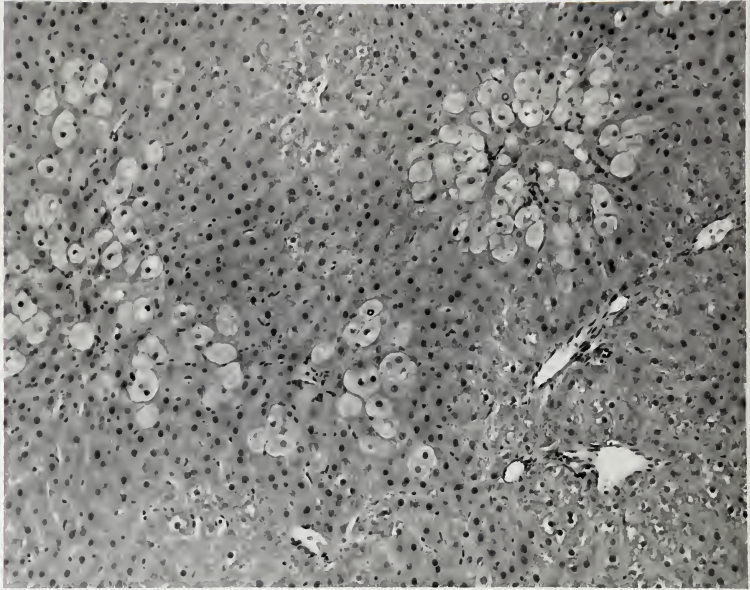


FIGURE viii-a

THE HISTOLOGICAL STRUCTURE OF THE LIVER
OF A RAT RECEIVING CORTISONE AND
CARBON TETRACHLORIDE

The following alterations are present:
centrolobular necrosis, focal necrosis, lobular
disarray, hydropic change (central), portal
inflammatory infiltrate, dispersed cytoplasmic
basophilia, regenerative activity and fatty
infiltration.

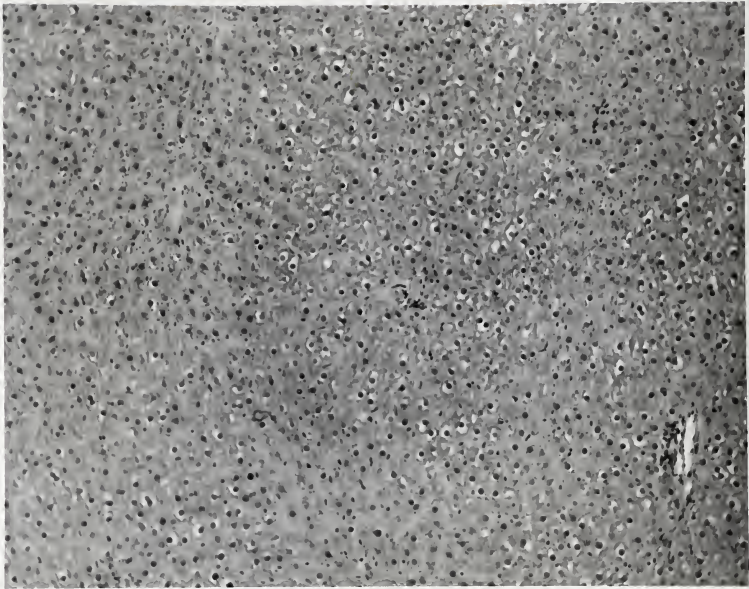


FIGURE ix

THE HISTOLOGICAL STRUCTURE OF THE LIVER
OF A RAT RECEIVING CORTISONE AFTER
CARBON TETRACHLORIDE DISCONTINUED

The following alterations are present:
binuclear cells, lobular disarray and
regenerative activity.

(magnification 20X)

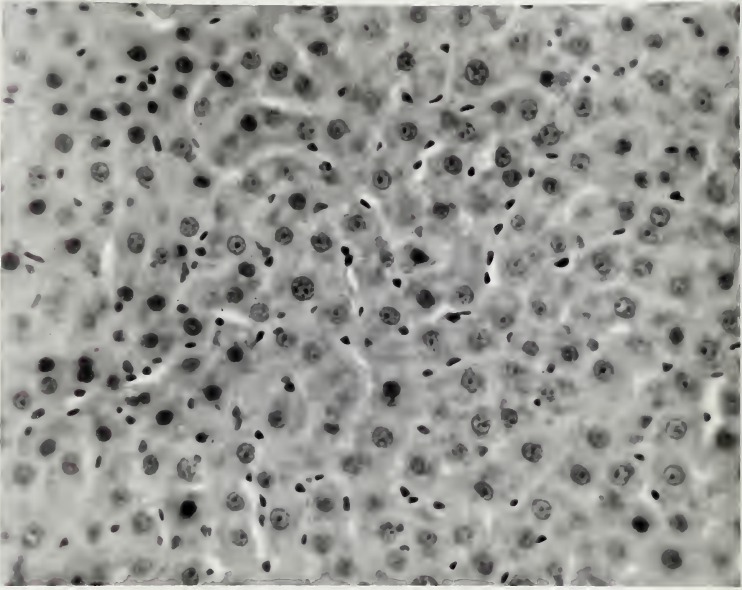


FIGURE x

THE HISTOLOGICAL STRUCTURE OF THE LIVER
OF A NORMAL RAT RECEIVING ALCOHOL

The liver appears normal.

(magnification 225X)

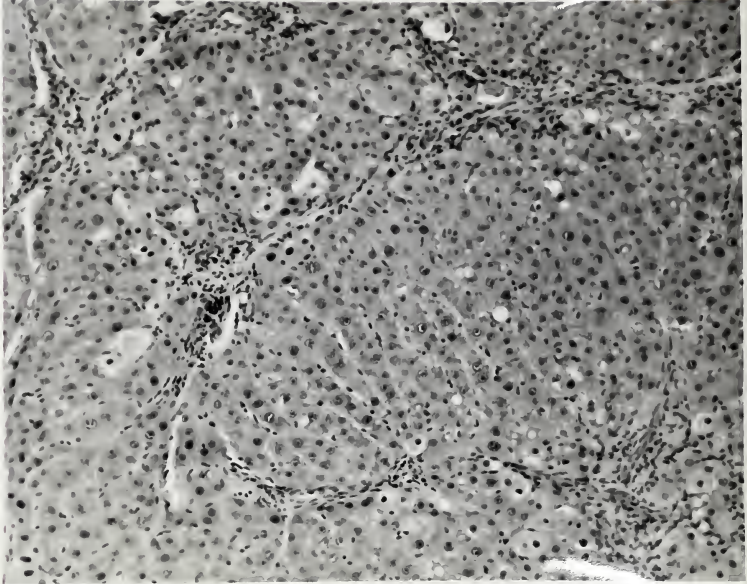


FIGURE xi

THE HISTOLOGICAL STRUCTURE OF THE LIVER OF
A RAT RECEIVING ALCOHOL AND CARBON
TETRACHLORIDE

The following alterations are distinctive:
portal inflammatory infiltrate, binuclear cells,
lobular disarray, and regenerative activity.
Other changes present are cirrhosis, fatty
infiltration, hydropic change (central),
centrolobular necrosis and focal necrosis.

(magnification 80X)

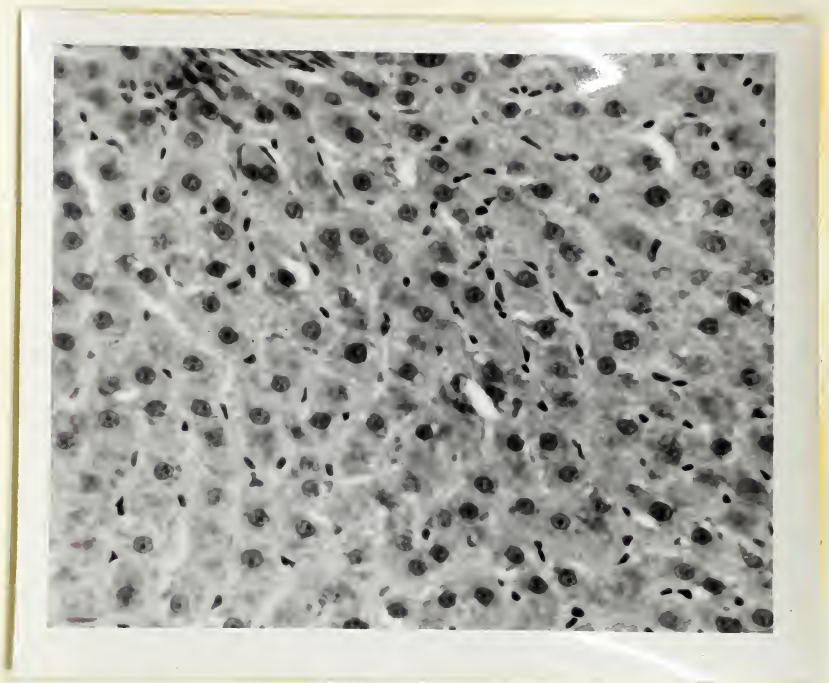


FIGURE xii

THE HISTOLOGICAL STRUCTURE OF THE LIVER OF
A RAT RECEIVING ALCOHOL AFTER CARBON
TETRACHLORIDE DISCONTINUED

The following alterations are present: binuclear
cells and regenerative activity.

(magnification 225X)

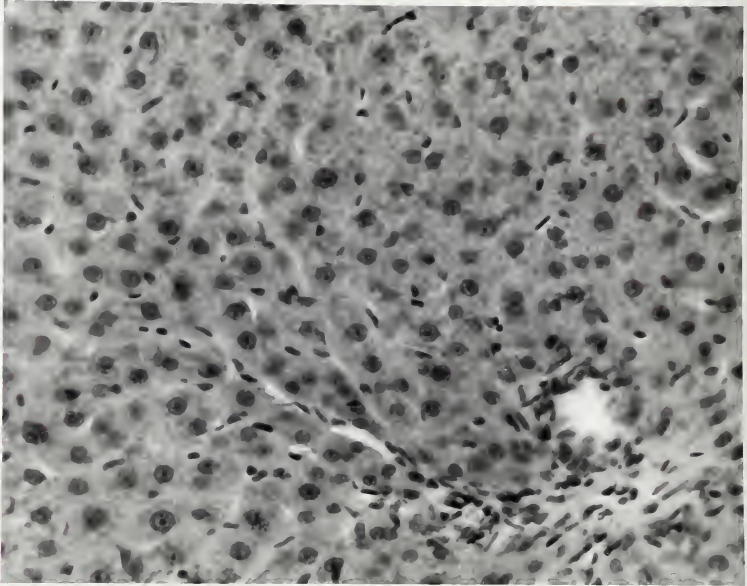


FIGURE xiii

THE HISTOLOGICAL STRUCTURE OF THE LIVER OF
A NORMAL RAT RECEIVING METHIONINE

The liver appears normal.

(magnification 80X)

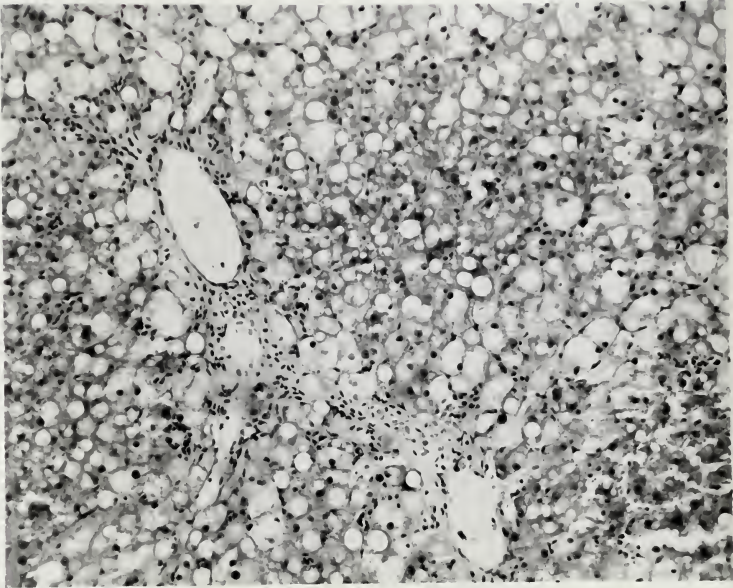


FIGURE xiv

THE HISTOLOGICAL STRUCTURE OF THE LIVER
OF A RAT RECEIVING METHIONINE AND
CARBON TETRACHLORIDE

The following alterations are distinctive:
fatty change, regenerative activity, portal
inflammatory infiltrate, and lobular disaray.
Other changes present are centrolobular necrosis,
focal necrosis, binuclear cells, dispersion of
cytoplasmic basophilia, and cirrhosis.

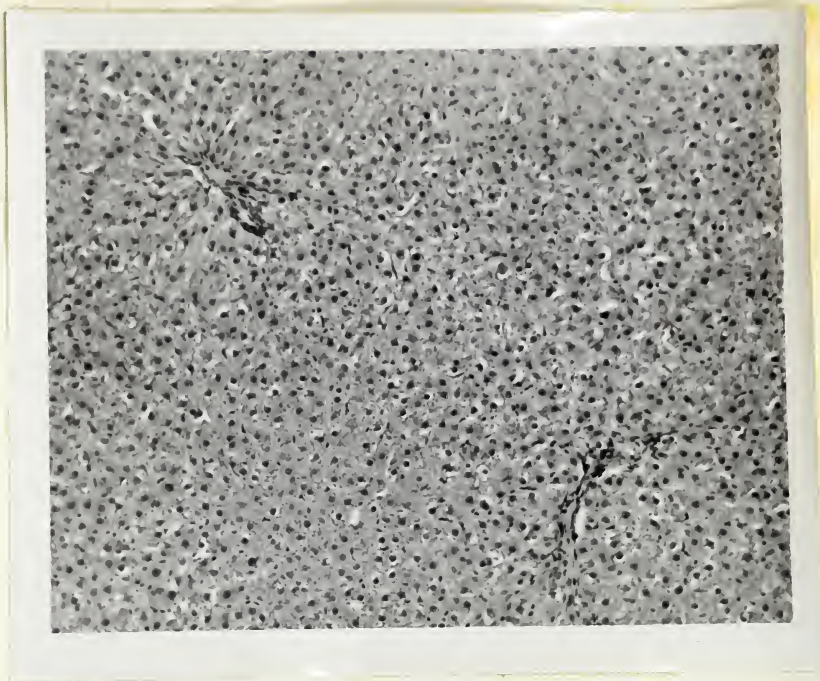


FIGURE xv

THE HISTOLOGICAL STRUCTURE OF THE LIVER OF
A RAT RECEIVING METHIONINE AFTER
CARBON TETRACHLORIDE DISCONTINUED

The following alterations are present:
binuclear cells, lobular disarray, regenerative
activity, and cirrhosis.

(magnification 80X)

SAMPLE CALCULATIONS SHOWING THE METHOD
OF DETERMINATION OF THE STATISTICAL
SIGNIFICANCE OF THE DATA OBTAINED
BY MEANS OF TISSUE RESPIRATION

Calculations for Respiration of Rats Receiving
Carbon Tetrachloride, Measured in HM Flasks

Days	QO ₂			
<u>x</u>	<u>y</u>	<u>xy</u>	<u>x²</u>	<u>y²</u>
1	2.62	2.62	1	6.8644
1	1.45	1.45	1	2.1025
8	3.12	24.96	64	9.7344
8	3.18	25.44	64	10.1124
.
.
.
.
<u>Sum</u>	<u>1522</u>	<u>136.07</u>	<u>4,857.97</u>	<u>63,698.</u>
			<u>63,698.</u>	<u>442.5985</u>

$$N = 44$$

$$\bar{x} = \frac{Ex}{N} = 34.59 \quad \bar{y} = \frac{Ey}{N} = 3.0925$$

$$s_{xy} = \frac{Exy}{N} - \bar{x} \bar{y} = \frac{4,857.97}{44} - (34.59)(3.0925) = 3.4384$$

$$s_x^2 = \frac{Ex^2}{N} - (\bar{x})^2 = \frac{63,698}{44} - (34.59)^2 = 251.21$$

$$s_y^2 = \frac{Ey^2}{N} - (\bar{y})^2 = \frac{442.5985}{44} - (3.0925)^2 = 0.495494$$

$$s_y = 0.7039$$

$$b = \frac{s_{xy}}{s_x^2} = 1.3687$$

$$b' = \frac{s_{xy}}{s_y^2} = 0.0694$$

$$r^2 = bb' = 0.09499 \quad r = 0.3082$$

Normal* values determined in a similar manner:

$$N^* = 57$$

$$\bar{x}^* = 55.44 \quad \bar{y}^* = 3.136$$

$$s_x^{2*} = 630.27 \quad s_y^{2*} = 0.1632$$

$$b^* = 0.4952$$

$$r^{2*} = 0.09468 \quad r^* = 0.3077$$

A. Student's t test for determination of significance of difference of means

$$t = \frac{\bar{x}^* - \bar{x} - (u^* - u)}{\left(\frac{N s_y^2 + N s_y^{2*}}{n + n^*} \times \frac{N + N^*}{NN^*} \right)^{\frac{1}{2}}}$$

where u = population from which sample taken
n = number of degrees of freedom

$$t = \frac{3.14 - 3.09 - (u - u^*)}{\left(\frac{57(0.1632) + 44(0.4952)}{56 + 42} \times \frac{55 + 44}{57 \times 44} \right)^{\frac{1}{2}}}$$

At the 95% level $t = \pm 1.96$

$$\pm 1.96 (0.0357) = 0.05 - (u - u^*)$$

$$(u - u^*) = 0.05 \pm 0.06997$$

Since these limits include zero, at the 95% level of significance there is no significant difference between the means.

Therefore there is no significant difference between the mean QO_2 values for normal and carbon tetra-chloride treated rats.

- B. Student's t test to determine the significance of the regression coefficient (b).

$$t = (b - B) \frac{s_x}{s_y} \left(\frac{N - 2}{1 - r^2} \right)^{\frac{1}{2}}$$

$$= (b - B) \frac{r}{b} \left(\frac{N - 2}{1 - r^2} \right)^{\frac{1}{2}}$$

where B = true regression coefficient in the population from which the sample is taken.

In order to determine whether an observed value of b differs significantly from zero, use

$$B = b \pm \frac{b}{r} (t) \left(\frac{1 - r^2}{N - 2} \right)^{\frac{1}{2}}$$

$$B = 1.3687 \pm \frac{1.3687}{0.3082} (1.96) \left(\frac{1 - 0.0950}{42} \right)^{\frac{1}{2}}$$

$$B = 1.3687 \pm 1.2435$$

Since the values do not include zero, the value of b is significant.

Therefore the QO_2 variable is directly dependent upon the length of time of administration of carbon tetrachloride.

- C. Student's t test to determine the significance of the difference between the slopes of the regression lines

$$t = \frac{b^* - b - (B^* - B)}{\left(\frac{s_y^2 (1 - r^2)}{(n - 2)(s_x^2)} + \frac{s_y^{2*} (1 - r^{2*})}{(N^* - 2)(s_x^{2*})} \right)^{\frac{1}{2}}}$$

$$t = \frac{0.4952 - 1.3687 - (B^* - B)}{\left(\frac{0.1632(1 - 0.0947)}{55(630.27)} + \frac{0.49459(1 - 0.0950)}{42(251.21)} \right)^{\frac{1}{2}}}$$

$$\pm 1.96 = \frac{-0.8735 - (B^* - B)}{2.071}$$

$$B^* - B = \pm 1.96(2.071) + 0.8735$$

Since these limits include zero, the difference between the slopes is non-significant.

Therefore there is no significant difference between the respiration of the normal and the carbon tetrachloride-treated animals.

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